BACTERIAL MODIFICATION OF DOUGLAS FIR ROUNDWOOD PERMEABILITY

A thesis

submitted in partial fulfilment

of the requirements for the Degree

of

Doctor of Philosophy

in the

University of Canterbury

by

Kevin John Archer

University of Canterbury

Christchurch, New Zealand

December 1985

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ABSTRACT

Douglas fir roundwood in New Zealand is currently not used in high decay situations because the heartwood is not sufficiently durable and the sapwood is not permeable to water-borne preservatives. The results in this study have confirmed that during water-storage of wood, bacteria selectively attack pit membranes, improving permeability, which leads to enhanced preservative uptake. To optimise conditions for bacterial growth and distribution within wood, the effects of temperature, nutrient supplements, pH and incising/kerfing were investigated during the sprinkling of short Douglas fir bolts in small tanks.

For water-sprinkling to be commercially acceptable the treatment time must be as brief as possible. Bacterial access to the wood is an important limiting factor. Tangential movement of bacteria in wood was found to be more rapid than radial movement and so the effect of incising or kerfing on bacterial access was investigated. Kerfing provided an ideal pathway for tangential entry into wood. Nutrient supplements in the sprinkling solution, particularly nitrogen and phosphate together, enhanced bacterial growth and enzyme activity, reducing the time required to achieve total sapwood preservative penetration. Sprinkling had no effect on heartwood permeability.

Sprinkling treatments also influenced the drying characteristics of Douglas fir. Short sprinkling times (1-4 weeks) reduced radial and tangential drying rates. Longer sprinkling times improved the rate of drying. Shrinkage measurements were made on small cubes cut from sprinkled

wood. Tangential shrinkage was reduced but radial shrinkage was unaffected; this should reduce the incidence of checking in water-sprinkled wood.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 PRESERVATIVE TREATMENT OF ROUNDWOOD

Wood, in contrast to concrete or steel, is considered perishable because of its susceptibility to biological deterioration. The principal agents of wood deterioration are decay fungi, which given the right combination of moisture, temperature and aeration, rapidly colonise susceptible timber. The heartwoods of some timbers are naturally resistant to decay due to the presence of chemical extractives laid down during heartwood formation. However, durability is not a feature of sapwood.

Provided that timber is kept dry, fungal decay does not occur. In certain situations the exclusion of water from wood is practical but for wood in ground contact it is rarely possible. Wood in ground contact is inevitably exposed to a high decay hazard. If cost were unimportant, perishable sapwood could simply be discarded before the timber was put into use but this is seldom economic. Indeed, reliance on the natural durability of heartwood will often not guarantee an adequate lifespan for a timber product anyway. To lower costs for the consumer and to conserve rapidly dwindling indigenous forest resources some means of preventing decay must be employed. The most simple, effective method available involves the use of chemical

preservatives.

The efficacy of a given preservative treatment depends on the amount of preservative absorbed (the retention) and the depth to which it penetrates. Wood exposed to a high decay hazard must have a higher preservative retention than that used in less severe conditions. Retention is a function of the wood treatability and also of the preservative concentration used. The preservative treatability of timber varies widely among different wood species and has led to the grouping of timbers into three categories: difficult to treat (refractory), moderately treatable and easily treated (McQuire et al. 1979). Hence treatment effectiveness depends ultimately on the wood's treatability.

A wood species is selected for a particular use on the basis of properties such as density, strength and growth form. The physical requirements for roundwood used as transmission line poles and building poles are often exacting. Correct taper, straightness, adequate bending strength, stiffness and small knot size are all requirements of a good pole. Many timber species fulfil those criteria but poor preservative treatability precludes their use as roundwood in ground contact situations. In some species presently used as poles, for example Scots pine (Pinus sylvestris L.), the heartwood is naturally durable and treatment of sapwood is all that is necessary to guarantee resistance to decay. Other species, for example Sitka spruce (Picea sitchensis (Bong.) Carr.)are not widely used for poles because the heartwood is non-durable and sapwood permeability is insufficient to allow adequate preservative

penetration. Until recently there has been little need to utilise such refractory species for poles. This is changing in view of projected worldwide shortages of durable and easily treated species. One simple solution would be to relax many of the exacting standards applicable to pole production. Extreme straightness and strength can be compromised in some situations, although it is important to maintain quality control to counter competition from reinforced concrete and steel alternatives. A more sound approach is to devise a means of improving the treatability of otherwise suitable refractory species. The latter approach is of considerable interest to countries such as Great Britain, keen to utilise her major plantation species, Sitka spruce, and so avoid expensive imports of Scandinavian Scots pine.

1.2 THE NEW ZEALAND SITUATION

Domestic stressed-pole requirements in New Zealand have traditionally been filled by Corsican pine (Pinus nigra Arnold). Where the physical attributes of roundwood are not so critical, for example with agricultural and horticultural fence posts, the demand has been filled by production thinnings from several species, mainly radiata pine (Pinus radiata D. Don). The demand for fence posts is high and is expected to remain that way in the forseeable future (Hellawell 1981). Stocks of Corsican pine have been dwindling due to the ravages of Dothistroma needle blight (Manley and Calderon 1982) and also to a Forest Service policy of not replenishing this species. The disease is not

prevalent in the Canterbury and Southland regions of New Zealand and as a result future plantings of Corsican pine will be confined to those regions; supplies will consequently be limited. Radiata pine is an obvious replacement species for stressed pole production, chiefly because of its availability. However, radiata grown in many parts of New Zealand is a low-density wood and does not produce strong poles. Moreover, extensive machining is required to remove the pronounced nodal swelling typical of radiata trees and that also reduces the strength of the finished pole. Stands of radiata pine can produce adequate poles but a silvicultural regime specifically for pole production is necessary. Such a regime conflicts with the present emphasis on clearwood sawlog production (Hellawell 1981). The desirability of specialised silvicultural regimes for radiata pine is very much dependent on the projected demand for poles (Manley and Calderon 1982). It is possible that alternative species not requiring the same intensive silviculture methods may be more economic.

Douglas fir (Pseudotsuga menziesii (Mirb.) Franco.) does not suffer from the same deficiencies as radiata and in many respects would make an ideal alternative to Corsican pine. Indeed, it is the main pole species grown in the Northwest of the U.S.A. The strength and form of Douglas fir round wood makes it ideally suited for pole use. It is the second most widely planted exotic species after radiata pine and in 1982 comprised 8% of the total timber cut (NZ Official Yearbook 1983). Under present New Zealand Forest Service silvicultural practice, the cost of producing high quality radiata pine saw logs can be partially offset by the

utilisation of production thinnings for fence posts and small poles. In contrast, Douglas fir thinnings are not normally harvested because the timber is refractory. This makes Douglas fir saw logs more expensive to grow. Douglas fir, like radiata pine, is non-durable (McQuire et al. 1979). Some form of preservative treatment is necessary before either timber can be used in high decay hazard situations. Radiata pine can be readily treated with water-borne preservative formulations but Douglas fir cannot. Vinden et al. (1979, unpublished) classified New Zealand grown Douglas fir in round post form as impermeable to water-borne preservative formulations. Moderately successful preservative treatment of Douglas fir sapwood is possible with oil-based preservatives but treatment plants with this capability are becoming rare in New Zealand (McQuire 1975). This is because oil-based preservatives are not only expensive (in part due to short supply) but they are also less environmentally acceptable than water-borne formulations. Preservative treatment of Douglas fir heartwood remains an intractable problem even with oil-based formulations. The great potential of Douglas fir as a stressed-pole commodity for the New Zealand market depends on the development of a process to improve its treatability.

1.3 IMPROVING THE PRESERVATIVE TREATABILITY OF REFRACTORY TIMBERS

The impregnation of wood with preservatives is influenced by three major factors; the anatomy of the wood being treated, its pre-preservative treatment history and

the preservation process itself. Variation in treatability among wood species has already been discussed but the problem is further compounded in refractory species because of extreme variability among individual trees. Logs are not treated individually, which means that any treatment 'charge' of refractory timber will contain wood with vastly different treatabilities. To avoid under-treatment it is generally necessary to treat to a higher preservative retention. Some logs will be over-treated while others may only just meet the required retention.

The problem of treating refractory timbers has been tackled in one of two ways:

[1] by increasing the permeability of the wood and

[2] by using more penetrating preservatives.

Wood anatomy, which determines permeability, is essentially a constant associated with the species under treatment. Improvements in permeability can result from a variety of pre-treatment procedures, but by far the simplest approach available to the wood preserver is the manipulation of the preservative solution and the means of applying that solution i.e. point [2] above.

1.3.1 Alteration of preservative treatment procedures

Hartford (1973) segregates the chemicals available to wood preservers into four major categories.

[1] Creosote, coal tar and related products

[2] Light organic solvents (strictly speaking,not a preservative as such)

[3] Oil-borne preservatives (pentachlorophenol and organotin compounds)

[4] Water-borne preservatives (Copper chrome arsenate CCA, ammoniacal copper arsenate ACA)

These materials are separated on the basis of their chemical composition. However, in terms of their behaviour inside wood tissue, only two major types of preservative are recognised: oil-borne preservatives and water-borne preservatives.

The impregnation of wood by these preservatives using pressure processes has proved to be a most effective means of achieving deep and uniform preservative penetration in refractory species. In general, the greater the applied pressure the greater the depth of penetration; the duration of the pressure period has also been shown to be an important variable (Hunt and Garratt 1967). However, the pressure cannot be increased beyond certain limits without causing wood to check or collapse. The maximum 'safe' pressure is species dependent, but in practice rarely exceeds 1400 kPa. Pressures up to 6000 kPa have been used successfully in Australia with some refractory hardwoods (Richardson 1978).

Both oil-borne and water-borne preservatives are amenable to pressure treatment, but a fundamental chemical difference between the two types affects their ease of penetration and maximum retention. Creosotes and oil-borne preservatives are non-polar chemicals which can occupy only the void spaces in treated timber. They do not cause

dimensional changes in treated products. The polarity of water-borne preservatives allows them to be absorbed within the wood cell walls as well as filling the void spaces. Nicholas (1972) observed that non-polar solvents penetrated wood more rapidly than polar solvents. He explained the observation by suggesting that the flow of a polar solvent such as water is impeded by its ability to form hydrogen bonds with the wood. Polar chemicals may not flow so fast, but their ability to penetrate the cell wall allows a greater retention than is possible with non-polar chemicals (Hunt and Garratt 1967).

The viscosity of a preservative affects its penetration into timber. High temperatures lower the viscosity of oil-borne formulations and that has been exploited in the treatment of refractory species. However, the temperature cannot be too high because wood becomes plastic and is more susceptible to deformation under pressure (Purslow 1974). There is no advantage in using elevated temperatures with water-borne preservatives because viscosity is not a limiting factor. Too high a temperature can cause massive precipitation of the preservative salts on the surface and outer layers of the timber, creating a physical blockage which prevents further uptake (Hartford 1973). The 'CELLON' process, used with some success on refractory species such as Douglas fir, takes advantage of the low viscosity of light hydrocarbon solvents, for example, liquid petroleum gas (LPG), to carry the preservative into the timber. The solvent rapidly volatilises after treatment, leaving the preservative (usually pentachlorophenol) behind. The preservative does

not migrate in service and the process is a completely 'clean' treatment (Arsenault 1973).

So far only methods for improving the penetrability of oil-borne preservatives have been discussed. Alternative solvent systems for inorganic salt preservatives have also been investigated. Anhydrous liquid ammonia (ALA) is one solvent found to be more effective at penetrating wood than water is (Rak 1977). ALA penetrates all components of wood cell walls, rapidly carrying with it dissolved preservative salts. Side effects such as cell wall plasticisation and minor microstructural damage accompany this penetration, but for most purposes those are insignificant. Initial successes with ALA led to the incorporation of ammonia into a number of aqueous preservative formulations, notably ammoniacal copper arsenate (ACA) and more recently copper-arsenic additive (CAA) and copper zinc-arsenate additive (CZAA), which have better fixation properties and greater fungal toxicity than ACA (Ralph and Shields 1984). Ammonia is a more expensive solvent than water and pressure treatment times are longer than with conventional procedures. Hence it remains to be seen whether ammonia-based preservatives are cost effective.

Many of the problems of pressure treating refractory species are associated with changes in the wood structure which occur during drying. Green wood is more permeable than dried wood and a number of preservation techniques take advantage of this. A major advantage of treating green timber is that the danger of fungal degradation which can occur during air drying is minimised. One of these techniques, the 'Boucherie' process, aims to displace the

sap in a freshly felled log with an aqueous preservative solution. In its most rudimentary form, a water tight cap is attached to the end of a log and preservative is forced in by hydrostatic pressure (Henry 1973, Purslow 1974). There are a number of variants of the process with various combinations of vacuum and pressure. The process can be time consuming and several days may be required to achieve adequate sapwood penetration. Careful filtering of the preservative solution is a prerequisite for a successful treatment. No treatment of heartwood is possible.

Methods which rely on the ability of inorganic salt preservatives to diffuse into green timber have proved useful in the treatment of refractory round wood. Green timber is immersed in a concentrated solution of preservative, removed after a short time and then kept moist for a number of weeks to allow sufficient time for the preservative to diffuse into the timber. Early problems with the premature fixation of preservative components have been resolved by double diffusion treatments (Vinden 1984 (a),(b)).

The oscillating pressure method (OPM) and alternating pressure method (APM) utilise vacuum and pressure to impregnate green timber. Both methods rely on the fact that not all of the void space in green timber is filled with sap; substantial air spaces exist. When green timber is immersed inside a pressure cylinder and subjected to alternating pressure and vacuum cycles, the sap and trapped air is exchanged with the preservative (McQuire 1964).

1.3.2 Physical pre-treatments

Physical pre-treatments alter the anatomy of refractory timbers to improve the depth of penetration and retention of preservative. This is especially true for the treatment of roundwood.

In the treatment of roundwood for ground contact it is necessary to ensure that the sapwood band is fully penetrated, providing a protective envelope around the untreated heartwood. Total sapwood penetration is desirable because roundwood in service has a tendency to check under drying stresses. The problem is severe in poles treated with water-borne preservatives because the wood has a high moisture content after treatment. Problems with checking can occur with large diameter poles treated with oil-borne preservatives, not because of a high moisture content after treatment, but because pre-treatment drying is often stopped when the moisture content of the outer sapwood zone is suitable for preservative treatment. Loss of moisture from inside the wood in service can lead to drying stresses and deep checking. If inner sapwood zones are not adequately treated then they may be exposed by checking and premature decay can result. Incising is an effective means of alleviating the stresses which cause checking and it also increases the depth of preservative penetration. For many species such as Douglas fir, adequate penetration of preservative would not be possible without incising (Perrin 1978). Incising takes advantage of the fact that wood under pressure conducts liquid more effectively in an axial direction than in a lateral direction from an exposed

surface. Careful arrangement of the incisions ensures that uniform preservative distribution is obtained by axial penetration alone. Incisions are generally made by mechanically forcing knives or needles into the timber surface. Inevitably the technique damages the wood surface and causes some loss in the modulus of rupture. The strength loss depends on the proportion of the total cross-section damaged (Banks 1973(a),(b)), but in roundwood it is generally a minor consideration when weighed against the increase in durability provided by the greater preservative penetration. Strength losses can be minimised through the use of high velocity liquid jets to incise timber because structural damage is reduced (Behr 1971). The energy requirements for liquid jets, however, make their use prohibitively expensive.

Deep incising is routinely used in the U.S.A. to pre-treat Douglas fir line poles at the ground line (Graham et al.1969, Graham 1973). A single saw kerf to the pith in conjunction with incising has been even more effective at reducing the severity of checking and thus preventing exposure of untreated wood to decay (Graham and Estep 1966, Graham 1973, Ruddick and Ross 1979). A recent trend in the treatment of refractory timbers is the use of incising in conjunction with alternative preservative solutions. Gjovik (1983) and Krzyzewski (1977) report on the advantages of combining incising with ammoniacal preservatives. Henshaw and Williams (1975) showed improvements in the retention and penetration of organic solvent wood preservatives in a Picea sp. after incising.

Steaming is another pre-treatment procedure which has

been moderately successful at improving treatability. The technique has two major advantages:

[1] It removes moisture from the timber, thereby decreasing drying times.

[2] The wood is more permeable than unsteamed timber because pit membranes and ray tissues are altered (Nicholas and Siau 1973).

Significant strength losses are associated with steaming timber, but the simplicity of the technique and its obvious benefits make it commercially acceptable.

1.3.3 Biological pre-treatments

Prevention of decay through wet storage of logs in ponds, lakes and rivers or by regular spraying with water has been practiced worldwide for many years, but particularly in the United States (Scheffer 1969). Logs stored in this manner are rapidly colonised by bacteria. The presence of those bacteria, combined with a saturated environment unfavourable to fungi, inhibits decay. Wet storage of large quantities of wood following catastrophic gales and fires has proved to be an invaluable means of preventing decay before the timber can be salvaged. The techniques and problems involved in setting up storage facilities have been reviewed by a number of authors (Boutelje 1976, Moltesen 1977, Clifton 1978, Liese 1984).

As well as preventing decay, water storage also increases the treatability of timber. Ironically, this was originally seen as a deleterious side effect. Over absorption of preservative by already permeable species was

a 'problem' and steps were taken to solve it (Ellwood and Ecklund 1959, Knuth and McCoy 1962, MacPeak 1963). The potential of water storage to improve the treatability of refractory timbers stimulated a great deal of research from the late 1950's to the mid 1970's. Literature reports on the subject are numerous and often repetitious. Some of those papers are worthy of note: Suolahti and Wallen (1958), Ellwood and Ecklund (1959), Knuth and McCoy (1962), Liese and Karnop (1968), Bauch et al. (1970), Banks (1970), Dunleavy and McQuire (1970), Unligil (1971) and Dunleavy et al. (1973). Much of the early work was concerned primarily with the cause and effect of permeability changes in water-stored wood; there was little emphasis on the factors controlling the phenomenon. Some logs were intentionally placed into water storage to examine specifically how treatability changed with storage time. In other cases, measurement of permeability was secondary to the primary purpose of assessing the incidence of decay.

Initially increases in treatability were thought to be due to fungal infections, particularly by Trichoderma spp. (Lindgren and Harvey 1952, Lindgren and Wright 1954, FPRL 1958). Schulz (1956) showed that the permeability of spruce and aspen could be increased after inoculation with Trichoderma spp. However, Johnson and Gjovik (1970) found that although Trichoderma viride alone could cause some change in permeability, the effects were negligible in comparison to those caused by a contaminating bacterium. They speculated that contamination by bacteria could account for the previous reports of improvements in permeability. Suolahti and Wallen (1958) and Ellwood and

Ecklund (1959) demonstrated that bacteria were responsible for permeability changes in water-storage environments. Knuth and McCoy (1962), following up the earlier work of Ellwood and Ecklund, isolated Bacillus polymyxa from over-porous pine. Using those isolates they inoculated sound pine and were able to increase its permeability after three weeks incubation at 30 °C.

Permeability changes are confined to the sapwood (Unligil 1971, 1972, Boutelje 1977). Bacterial colonisation of sapwood resulted in the loss of pectinaceous material primarily from the pit membranes (Suolahti and Wallen 1958). The crossfield pit membranes between tracheids and rays and the tori of bordered pits contain a high proportion of pectin (Liese 1970). It was suggested that this loss accounted for the observed improvement in permeability. Ellwood and Ecklund (1959) were able to relate excessive permeability to the depletion of ray cell contents and in severe cases complete dissolution of the ray cell walls. This was confirmed by Greaves (1965) working with sapwood cubes of Pinus radiata and Eucalyptus regnans. Using commercial pectinase enzymes and small cubes, Suolahti and Wallen (1958), Liese and Karnop (1968) and Dunleavy and Fogarty (1971) obtained permeability improvements similar to those occurring during water-storage. It was concluded that pectinolytic activity by bacteria was the cause of permeability improvement. However, Bauch et al. (1970) and Meyer (1974) found that enzyme treatments are not equally effective on all timbers. Douglas fir for example was less amenable to pectinase treatment than spruce and this was attributed to differences in the composition of the pit

membranes.

The association of bacterial enzymes with water-storage was investigated in a series of papers by Ward and Fogarty (1971,1973,1974) Fogarty and Ward (1973,1972(b)) and Dunleavy et al. (1973). High pectinase activity and xylanase activity were detected in sap squeezed from water-stored spruce. This was correlated with permeability improvement and loss of pit membrane material. Negligible cellulase activity was reported.

The effects of water-storage on permeability have been examined for a wide variety of different timber species. The results reported from those studies lack any cohesive pattern save for the fact that permeability is improved. This is partly due to the number of timber species used and their different behaviour in water storage, but it is also due to different interpretations of the phrase 'permeability improvement'. Some authors have assessed the effects of water storage from measurements of gas and liquid permeability in small samples cut from water-stored logs, others in terms of increases in preservative uptake in whole logs. There are problems involved in relating the permeability of small samples to whole logs and gas permeability to preservative treatability (Booker 1980(a) unpublished). A more fundamental problem when comparing the results reported by different studies is a lack of standardisation and control of the environmental parameters which influence water-storage. Since changes in permeability are mediated by bacteria, environmental factors which influence bacterial growth and metabolism are important (Bauch et al. 1970).

Bacteria with the ability to effect permeability changes must first colonise the wood; they are not natural inhabitants of standing timber. Colonisation of and migration into timber are slow processes. Minimum water storage times necessary to improve permeability range from a few weeks (Schulz 1968) to several months (FPRL 1958), but obviously those times are dependent on the water-storage environment.

The increase in permeability of pine wood stored in stagnant water was found to be three times greater in summer than in winter (Ellwood and Ecklund 1959). Lutz et al. (1966) reported that southern pine logs stored under water in summer months showed greater permeability than logs stored in winter or under water sprays for a comparable time. Liese and Karnop (1968) observed dramatic changes in the size of bacterial populations living in water-stored pine and spruce with the transition from winter to spring. Dunleavy (1973) and Dunleavy et al. (1973) confirmed that the effectiveness of spruce ponding depended on the temperature of the storage water. Banks (1970) made a thorough examination of the influence of temperature on wet-storage. Temperatures around 20°C promoted permeability improvement because bacterial growth was faster; at 10°C permeability changes were slower.

Logs stored submerged in stagnant ponds are subjected to an anaerobic environment, while those stored under water sprays or in moving water are exposed to more aerobic conditions. The influence of dissolved oxygen concentration on microbes responsible for permeability improvement during water storage was examined by Banks and Dearling

(1973). Using aerated and stagnant pond water in a laboratory-scale study, they concluded that the magnitude of any permeability change was the same in aerobic and anaerobic conditions, but the rate of improvement was faster in an aerobic environment. Whether or not the permeability changes were the result of the same organisms in the different conditions was unclear but their experiments indicated that the degree of aeration was an important factor in optimising the water-storage process.

Thus, by the early 1970's, water-storage was firmly established as a method capable of improving the permeability of refractory species. Liese (1984) discussed the commercial feasibilities of ponding and sprinkling as a means of preventing decay but his arguments are also relevant to permeability improvement. He suggested that ponding was less favourable than sprinkling for the following reasons:

[1] suitable ponds and lakes are not widely available,

[2] they hold only 1000m^3/ha compared to 10000m^3 under sprinklers,

[3] loading and unloading can cause technical problems,

[4] losses may occur due to sinkers,

[5] moisture contents in some stems floating above the water may be too low to prevent decay and

[6] water pollution problems result from bark leachates

For the technique to become commercially feasible it is necessary to optimise the process. Time is crucial

because of the financial penalties incurred by tying up capital for too long. Extended water-storage also lowers the quality of the wood slightly. Harmless degradation of pit membranes could extend to degradation of the cell wall itself, especially after long term exposure (Greaves 1969). Decay from other sources, especially soft rot fungi has also been reported during prolonged ponding (Unligil 1971,1972).

There have been a number of studies to establish the optimal conditions for bacteria in water-storage environments. Banks (1970) favoured the spraying of debarked logs with bacteria followed by air drying. In laboratory experiments, Fogarty and Ward (1972(b)) suggested that water storage would be most efficiently carried out in tanks inoculated with bacteria capable of producing large amounts of pectinase, rather than relying on opportunistic colonisation from a natural lake environment. They reasoned that the use of such an enclosed system would allow a higher level of pectinolytic activity to be maintained than would be possible in a lake or pond situation which is continually exposed to dilution from surrounding water. Dunleavy et al. (1973) also used enclosed tanks and compared the effects of using a natural bacterial inoculum from lake water against using bacteria with known pectin degrading ability. A comparison of ponding versus sprinkling was also made. The storage temperature was controlled at 20°C and the water circulated to provide adequate aeration. Increasing the water temperature increased the bacterial count considerably and so reduced the storage time required to improve permeability. It was found that enzyme

production paralleled bacterial growth. They concluded that any modification which induced earlier pectinase production or increased the total pectinase activity would reduce the time requirement for successful permeability improvement. An understanding of the factors which regulate the synthesis of the enzymes involved in permeability improvement is essential for the development of a bacteriological pre-treatment of wood prior to preservation (Kurowski and Dunleavy 1976(b)).

Pectinase activity in bacterial isolates from water-stored wood is generally due to the production of polygalacturonate acid transeliminase (PGTE) (Macken and Pickaver 1979). A number of bacterial isolates from water-stored wood have exhibited PGTE activity in culture. Kurowski and Dunleavy (1976(a)) found an inducible PGTE enzyme in cultures of Cytophaga johnsonii and a constitutive PGTE was produced by Bacillus subtilis (Kurowski and Dunleavy 1976(b)). They observed that the enzymes occurred both intracellularly and extracellularly but 99% of the activity occurred in cell free supernatants. PGTE will degrade only demethylated pectin (Albersheim et al. 1960), yet no pectin methyl-esterase production was detected. Lack of methyl-esterase activity was also observed in cultures of Enterobacter cloacae isolated from water-stored spruce by Macken and Pickaver (1979). They suggested that some other unidentified organism might be a pectin methyl-esterase producer assisting Enterobacter cloacae in degrading pectin. This possibility of synergistic activity was considered earlier by Fogarty and Ward (1973). They observed in mixed culture experiments that

the growth and enzyme production by Flavobacterium pectinovorum were enhanced in the presence of another organism, Bacillus subtilis. The use of mixed cultures in water-storage experiments was advocated by Dunleavy et al. (1973) who unsuccessfully tried to imitate natural ponding by seeding tanks with Bacillus cereus, B. circulans and a Cytophaga sp. Successional changes in the microflora of water stored spruce were followed by Macken and Pickaver (1979). On the basis of those results they stressed the need for care in the selection of bacterial species for reinoculation into a water storage environment.

The problems associated with the commercial application of water-storage to improve the permeability of refractory timbers have have been described by a number of authors. Boutelje (1976,1977) observed that the permeability of spruce to creosote could be improved markedly after ponding for 11 weeks in warm weather. The effect was greatest on poles that had been debarked before storage. However, the uptake results varied considerably from log to log. Boutelje suggested that conventional preservative treatment procedures were not satisfactory for ponded material. Krzyzewski (1979) investigated the industrial possibilities of ponding white spruce, Picea glauca (Moench) Voss and was able to demonstrate adequate preservative penetration for ground contact use after 12 weeks pond storage at 18°C. Pre-steaming before creosote impregnation resulted in some checking and honeycombing in the treated product. In agreement with Boutelje, Krzyzewski suggested that an alternative preservative treatment schedule may have prevented those defects. Once again the

best results were obtained on debarked poles. Krzyzewski found that ponding and sprinkling intact poles (complete with bark) was ineffective.

Fowlie (1981) reported on a large scale sprinkling trial to improve the treatability of Sitka spruce for use as line poles. Spruce logs were stored under water sprays seeded with 14 species of bacteria known to affect permeability. It was difficult to maintain temperatures suitable for bacterial growth and loss of moisture from evaporation was a constant problem. After 148 days sprinkling, none of the original inoculum could be re-isolated. Few poles sprinkled in the trial met the required preservative uptake and the variation among poles was high. In a later paper (Fowlie and Sheard 1983), the results from the earlier sprinkling trials were compared with those obtained from ponding replicate spruce material for 6-8 months. Preservative uptake was higher in the ponded material and penetration patterns for creosote and CCA were similar to one another. However, the authors considered that the 20 months which elapsed from felling to delivery (including a seasoning period after treatment with CCA) was excessive and uneconomic.

1.4 THE AIMS OF THIS STUDY

There is a need to improve the treatability of refractory timbers to supplement dwindling supplies of treatable species. Physical pre-treatments and the manipulation of preservative procedures have proved useful, but in general those techniques alone do not provide the

level of preservative treatment necessary for high decay hazard situations. This author believes that biological pre-treatments can succeed where the other techniques have failed. The purpose of this thesis is to examine the potential of biological pre-treatment to improve the treatability of New Zealand grown Douglas fir to CCA preservatives. There is little published information on the effects of water-storage on Douglas fir, but the few reports available indicate that the timber is less amenable to permeability improvement than other species are (FPRL 1958, Bauch et al. 1970, Meyer 1974).

Despite the number of investigations into the effects of water storage on permeability, the process still is poorly understood. One of the major limiting factors preventing the implementation of water-storage as a pre-treatment procedure is the length of time involved. If that time factor could be minimised, then water-storage might become economic. Two major factors affect the time requirement directly:

[1] the ecology of bacterial growth in water-stored wood and

[2] the ease of bacterial access into the wood

The influence of temperature and aeration on bacterial growth has been described previously, but the effects of nutrient levels on that growth have received minimal attention. Dunleavy et al. (1973) used a mineral salts medium in small scale ponding experiments, but other workers have tended to use natural pond water. Macken and Pickaver (1979) considered that micro-nutrients present in water were

important for fulfilling enzyme co-factor requirements. In this investigation the effects of added nutrients, temperature and aeration on Douglas fir placed under water sprinklers will be examined.

Studies on the microbial population inhabiting water-stored logs indicate that successional changes play an important part in permeability improvement. Attempts to inoculate freshly felled logs with selected organisms in enclosed tank systems have largely been unsuccessful (Fowlie 1981), yet this approach has much to offer in terms of optimising water-storage. It is important that any organisms used to 'seed' storage water be competitive; logs in water storage are far from sterile. The use of a single organism in water storage is not desirable. Wood is a non-homogeneous substrate and a wide range of enzymes capable of degrading pit membranes will most likely be found in a mixed bacterial population. This is in effect what occurs in nature. This study proposes to use such a mixed population of bacteria.

A problem frequently encountered with water-stored wood is the variability of subsequent preservative treatments, not only among different logs and but also within individual logs. It is likely that the cause of this variability is the poor accessibility of wood to bacteria. No permeability improvement occurs without bacteria, but only the outer growth rings in round wood are immediately accessible to colonising organisms. Migration of bacteria from the outside to the inside is a slow process. This fact has been largely ignored, but is fundamental to increasing the rate of permeability

improvement through biological means. The potential for mechanical incising and kerfing to open up the wood structure to bacteria will therefore be examined in this study.

CHAPTER 2

2.1 PERMEABILITY - A THEORETICAL DEFINITION

In the wood preservation industry timbers are classified as very permeable, permeable, resistant or very resistant depending on how easily they can be impregnated with preservatives using conventional treatment processes.

Permeability can be defined as a measure of how easily a fluid flows through a porous material under a pressure gradient. This definition can be quantified mathematically by Darcy's Law (Darcy 1856). Darcy's Law (equation 2-1) is valid only under steady-state conditions and when resistance to flow is due to viscous drag. In spite of these limitations, Darcy's Law serves to highlight those parameters important to an understanding of permeability in wood.

$$K = (Q \times U \times L) / A \times P \qquad (2\text{-}1)$$

where K = the permeability constant (m^2)
Q = fluid flow rate measured ($m^3 s^{-1}$)
U = viscosity of the fluid ($10^{-1} Nm^{-2}.s$)
L = length of the specimen in direction of flow (m)
A = cross sectional area perpendicular to the direction of flow (m^2)
P = pressure drop between the two ends of the specimen (Nm^{-2})

The rate of fluid flow through a porous medium varies according to the fluid's characteristics as well as the structure of the medium. The inclusion of the viscosity term 'U' makes 'K' independent of the permeating fluid and

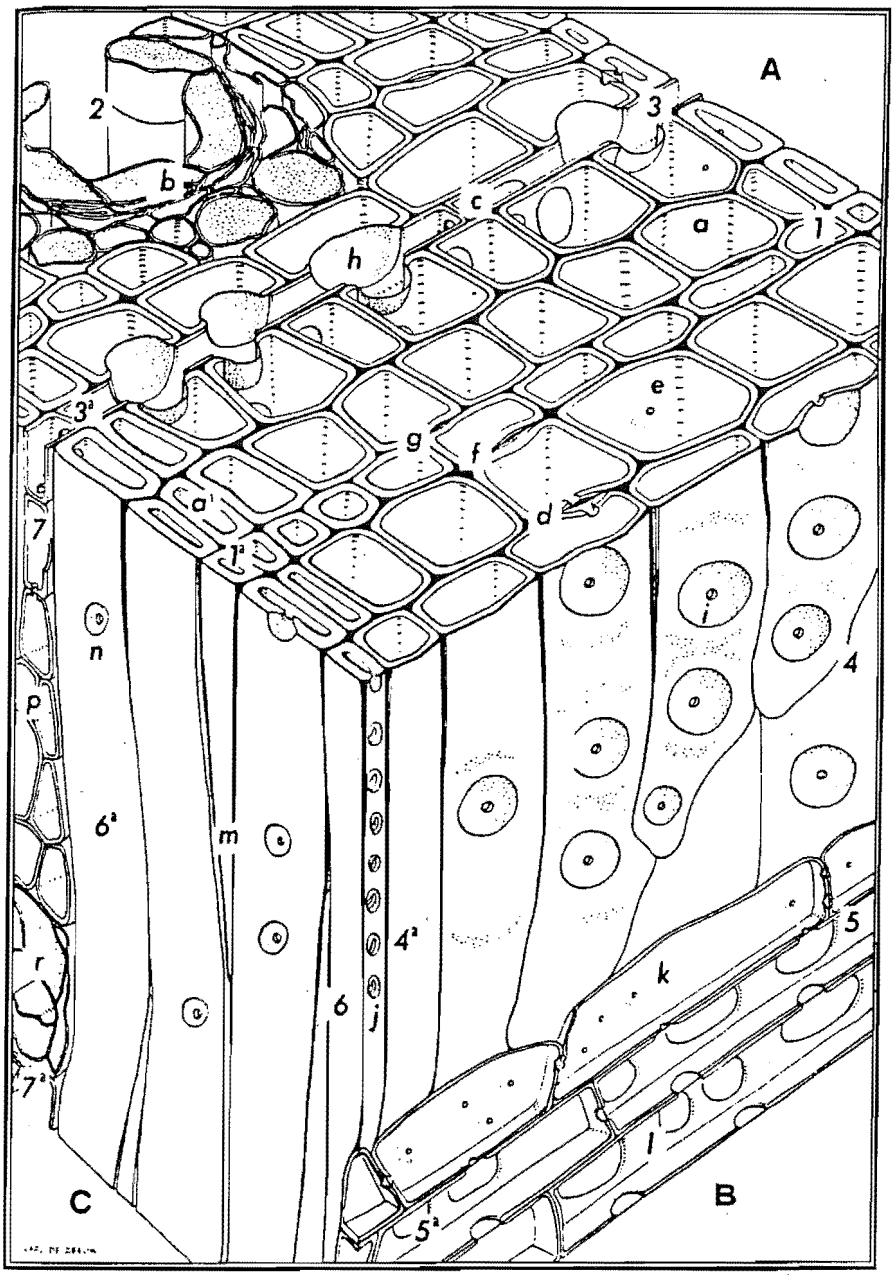
solely dependent on the medium. Equation 2-1 assumes that the fluid does not cause any physical or chemical changes in the porous medium.

2.2 THE IMPORTANCE OF WOOD ANATOMY TO PERMEABILITY

The pathways for the conduction of sap in the living tree can also function as pathways for preservative flow. These pathways are evident in Figure 2-1 which depicts a schematic, three dimensional diagram of a typical softwood.

Tracheids constitute the bulk of the volume of softwoods, some 90-94% on average (Panshin and de Zeeuw 1980, p.127), and function as vertical conduits for the flow of sap. Tracheids can be regarded as long, imperforate, narrow tubes with their ends tapered along their tangential walls. Over a considerable proportion of their length they are interconnected by bordered pits. Bordered pits are most numerous in earlywood, but occur in latewood also. The actual number of pits per tracheid varies with the tree species. Numbers usually range from 50 to 300 in the early wood, with much lower counts in the late wood (Siau 1971, p.15). Bordered pits are found mainly on the tangential walls, but a few occur on the radial walls of tracheids at the earlywood/latewood boundary. The orientation of vertical tracheids and the presence of pits on the radial walls precludes the possibility of the tracheids' functioning efficiently in radial flow in the living tree. Ray tissue is thought to fulfil this role. Rays occupy only 7% of the total volume of softwood timber

Figure 2-1 Schematic three dimensional diagram of a typical softwood. Source: Panshin and de Zeeuw (1980, p.129).



KEY:

Surface A. 1-1°, portion of an annual ring; 2, resin canal; 3-3°, wood ray; a-a', longitudinal tracheids; *b*, epithelial cells; c, ray cells; *d*, pit pair in median sectional view; bordered pits in the back walls of longitudinal tracheids, in surface view; *f*, pit pair in sectional view, showing the margin of the torus but so cut that the pit apertures are not included in the plane of section; *g*, pit pair in which neither the pit aperture nor the torus shows; *h*, window-like pit pairs between longitudinal tracheids and ray parenchyma.

Surface B. 4-4°, portions of longitudinal tracheids in radial aspect (the ends are blunt); 5-5°, upper part of a uniseriate ray; *i*, bordered pits on the radial walls of longitudinal, early-wood tracheids (the base of the pit is toward the observer); *j*, small bordered pits on the radial walls of longitudinal late-wood tracheids, in the same view as in *i*, *k*, ray tracheids; *l*, cells of ray parenchyma.

Surface C. 6-6°, portions of longitudinal tracheids in tangential aspect; 7-7°, portion of a xylary ray; *m*, tapering ends of longitudinal tracheids; *n*, a small bordered pit on the tangential wall of a longitudinal late-wood tracheid; *p*, cells of ray parenchyma; r, transverse resin canal.

(Panshin and de Zeeuw 1980, p.127) but they are so distributed that each vertical tracheid is in contact with at least one ray system. Two types of ray systems are recognised in conifers:

[1] homogeneous rays, consisting of short parenchymatous cells only, linked to one another through simple pits

[2] heterogeneous rays characterised by the presence of parenchyma, ray tracheids and in some cases horizontal resin canals.

Ray tracheids (case [2]) are linked to one another by bordered pits. Where ray tracheids are absent or poorly developed, there is a much higher incidence of bordered pits on the radial walls of the latewood tracheids (presumably to facilitate radial movement) (Bailey 1966 Thesis).

2.2.1 Cell wall pitting

A pit can be defined as a recess in the cell wall with an external closing membrane. Two major types of pits are observed in softwoods:

[1] simple pits
[2] more complex bordered pits

Normally two opposing pits in adjacent cells occur as a pit pair. Various combinations of bordered pits and simple pits occur giving rise to bordered pit pairs, simple pit pairs and half bordered pit pairs. In some cases single pits occur without a corresponding partner in an adjacent cell. This gives rise to blind pitting; blind pits often

adjoin intercellular spaces. The ultrastructure of bordered pit membranes has been examined extensively since the advent of the electron microscope (Liese 1965, Fengel 1972, Bauch et al. 1973, Meyer 1974, Tschernitz and Sachs 1975). Less is known about other types of membranes.

A schematic diagram of a bordered pit pair is presented in Figure 2-2. Bordered pits are circular

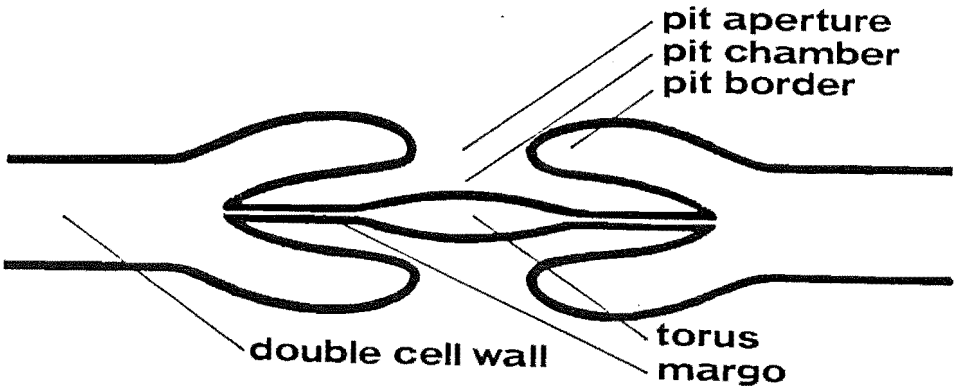


Figure 2-2 Schematic diagram of a bordered pit pair.

openings in cell walls and are spanned by a thin membrane which is a continuation of the middle lamella and the primary cell wall. The bordered pit membranes of some conifers have a central thickened region known as a torus. The torus is supported by strands of cellulose microfibrils (Bauch et al. 1968) usually referred to as the margo. Two types of microfibrils are recognised in most conifers; the first is a system of thin strands lying in all directions and the second a system of thicker, radially

orientated strands running from the torus to the edge of the pit. The two cross over each other creating a porous microfibrillar web (Liese 1965). The size of these pores greatly influences the permeability of the wood. The most accurate data on their size come from studies using wood to filter out different sized particles. Estimates of pore size depend on the species, but values between 0.01 µm and 4 µm have been reported (Siau 1971, p.17, Petty and Puritch 1970) for the maximum pore openings. It is doubtful that measurements taken from electron micrographs of bordered pit membranes are reliable. Surface tension during the drying of specimens for electron microscopy disturbs the original microfibrillar structure (Thomas and Nicholas 1966). Sachs and Kinney (1974) maintained that the classical picture of the bordered pit pair just described is a drying artifact. Nevertheless, fluids are able to flow through the fine pores in the margo, but the torus is regarded as being impermeable. The torus also has a microfibrillar substructure, but it is occluded by encrusting materials.

Considerable information on the chemical composition of bordered pits has been gained from studies of their development. In its most basic form, the bordered pit pair possesses a layered structure comprising the remnants of primary wall material from two adjacent tracheid cells and the middle lamella sandwiched in between (Krahmer and Cote 1963, Nicholas 1968, Tschernitz 1973, Tschernitz and Sachs 1975). The primary wall consists of randomly orientated cellulose microfibrils embedded in a matrix material composed of hemicellulose and polyuronides (Preston 1974). Matrix materials are deposited at the same time as

the cellulose microfibrils, that is they are distinct from encrusting materials (lignin and polyphenolic compounds) which are deposited later (Jurasek et al. 1967). In contrast, the middle lamella lacks any defined substructure. In the final stages of secondary wall development, the middle lamella becomes lignified except in the vicinity of pit fields. The central region of a pit field may become thickened by the deposition of further matrix materials (particularly polyuronides) and circularly orientated microfibrils on top of the primary wall forming the torus. During xylem cell development it is thought that the primary wall in the pit region is attacked by enzymes during autolysis of the cell and the matrix substances are removed, exposing the cellulose microfibrillar web and forming the margo (Barnett 1981, Thomas 1969). The torus is resistant to degradation. It has been suggested that the torus remains after cell autolysis simply because it is thicker than surrounding primary wall (Fengel 1972). A more likely explanation is that the wall is protected from enzyme attack by its polyuronide content (O'Brien 1970). Several investigations have demonstrated the susceptibility of the margo encrusting substances to hemicellulase enzymes but the torus shows a marked degree of resistance to those enzymes. However, the torus can be readily degraded by pectinase enzymes (Nicholas and Thomas 1968, Bauch et al. 1970, Imamura et al. 1974, Meyer 1974).

Simple pits between adjacent parenchyma cells are less complex. Essentially they are gaps in the secondary cell wall bridged by a loosely packed network of microfibrils encrusted with amorphous material. No central thickening of

the membrane can be seen and perhaps more important there are no microscopically visible pores to allow the passage of fluids. The membranes of half bordered pit pairs between axial tracheids and rays resemble the simple pit membranes closely but they are frequently thicker. The parenchyma side of the membrane is usually encrusted to such a degree that its surface structure is not visible (Kollman and Cote 1968, p.37). To the author's knowledge there have been no studies specifically concerned with the structure and chemical composition of the half-bordered and simple pits in Douglas fir. The meagre amount of information that is available is scattered over a multitude of publications. Douglas fir has thick walled ray cells in common with Picea spp. and Abies spp. (Imamura 1974). The half-bordered pit membrane consists of the primary wall of the tracheid, the middle lamella and the primary wall of the ray cell. Ray cell walls in Douglas fir have a type of secondary thickening which is absent from the pit areas giving the pit a recessed appearance when viewed from the ray side. Bailey (1936) and more recently Saka et al. (1981) have found that the ray tissues in Douglas fir are highly lignified, more so than the longitudinal tracheids. However, Bamber (1961) using histological stains, determined that the pit membranes themselves were not lignified although they were heavily encrusted. Krahmer and Cote (1963) were unable to determine the presence of any microfibrils in the membrane of Thuja plicata (of similar structure to Douglas fir) and attributed that to the presence of encrusting substances. Imamura (1974) examined the half-bordered pit in Chamaecyparis after hemicellulase and pectinase treatment. He observed that

hemicellulase removed much of the amorphous substances encrusting the membranes revealing an underlying fibrillar structure. Pectinase treatment degraded the central part of the membrane indicating a high pectin content.

2.3 PERMEABILITY AND PRESERVATIVE FLOW PATHS IN SOFTWOODS

It is well known that the permeability of wood varies markedly in its three principle structural directions; typically the ratio of longitudinal to tangential permeability varies from 520 to 81600 and longitudinal to radial from 15 to 547000 depending on the species (Comstock 1970). Most of these differences can be simply explained by the anatomical anisotropy of wood. Although axial flow is known to be greater than transverse flow, timber for preservative treatment is generally much longer in the axial direction than in transverse directions; thus transverse flow is of greater importance to the penetration and distribution of preservatives during preservative treatment.

The bulk of fluid transport in the axial direction occurs from tracheid to tracheid via the bordered pits (Erickson and Crawford 1959, Wardrop and Davies 1961, Erickson and Balatinecz 1964, Bailey and Preston 1969 and 1970). Bordered pits also facilitate tangential flow but their importance to radial flow is unclear. Ray tissues are more ideally placed to allow radial flow.

There is considerable confusion in the literature associated with attempts to relate permeability to subsequent preservative treatability. The terms are far from being synonymous, although in most cases a highly permeable

wood will treat more successfully than a wood with low permeability (Tesoro and Choong 1976). Some workers have attempted to relate permeability to subsequent preservative uptake (Erickson and Estep 1962, Siau and Shaw 1971, Tesoro and Choong 1976, Murmanis and Chudnoff 1979). Unfortunately those efforts have been only partly successful. One major reason for the lack of success is that the natural flow paths for fluids are altered by both drying and the pressures used in preservative treatment. Nonetheless, Siau and Shaw (1971) stated that the air permeability of wood, irrespective of the species, was the most accurate parameter for predicting preservative treatability.

2.3.1 Pit aspiration

In general, timber needs to be dried to just below fibre saturation to achieve maximum penetration and retention of preservative. Yet it is well known that the permeability of wood decreases as it dries. That reduction in permeability is attributed to pit aspiration (Comstock and Cote 1968). When aspiration occurs, axial and tangential permeability are low; if aspiration is prevented then axial and tangential permeability remain high.

In green wood, most of the sapwood pit membranes are centrally located and quite permeable. Surface tension forces during drying displace the membrane, causing it to come into contact with the pit border. Generally once contact has been made, the torus adheres to the pit border by hydrogen bonding (Figure 2-3), sealing off the pit and preventing fluid flow (Petty 1972, Thomas and Kringstad

1971). Pit displacement has also been shown to occur as a result of pressure treatment and it is thought that this

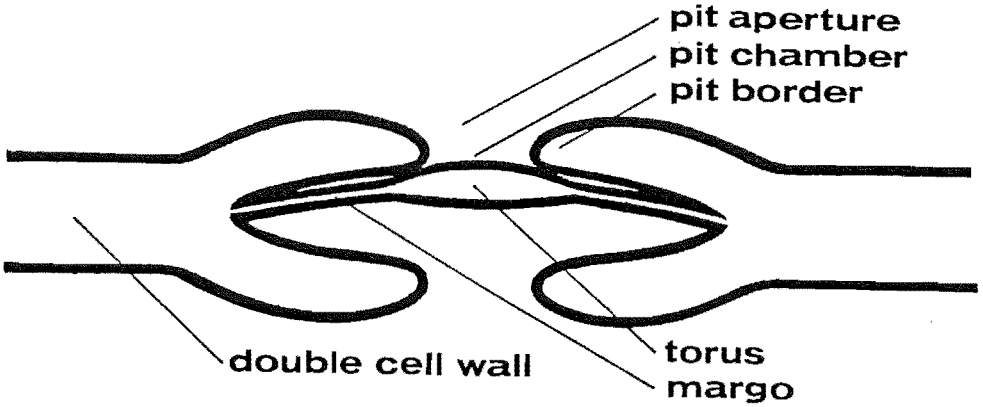


Figure 2-3 Schematic diagram of an aspirated bordered pit.

observation explains in part some of the deviations from Darcy's Law, which predicts that the amount of flow is directly proportional to the pressure applied. In practice the relationship of fluid flow to the applied pressure is far from linear and decreasing flow rates are often observed under constant pressure. The extent to which the torus or pit membrane is displaced (and hence the flow reduced) is proportional to the pressure applied (Bailey and Preston 1970). Partial deaspiration of pit membranes does occur after resoaking in water. The frequency of deaspiration after drying depends on the length of time that the pits were aspirated and also the length of the soaking time (Thomas and Kringstad 1971).

A study of the flow pathways in the living tree

suggests that the permeability of earlywood is greater than that of latewood. It seems, however, that the opposite is true in dried wood, although the evidence is somewhat conflicting (Erickson and Estep 1962). Both air and kiln drying cause the aspiration of all earlywood bordered pits but the much smaller latewood pits resist aspiration. Evidently this is because the latewood pit membranes are more rigid and the cell walls are much thicker, therefore a greater force is required to aspirate the pit (Liese 1965, Comstock and Cote 1968, Petty 1972). In earlywood, the force generated by surface tension greatly exceeds the mechanical resistance of the pit membrane and so it aspirates. Aspiration of the torus in earlywood requires extension of the margo strands by as little as 8% of their original length. In contrast aspiration of latewood bordered pit tori requires a 28% extension because of the thicker cell wall (Petty 1972). For this reason latewood is generally found to be more permeable than earlywood after seasoning (Petty and Preston 1969(b)). Ray tracheid bordered pits are similar in size and structure to the latewood pits and exhibit the same resistance to aspiration.

Bordered pits are able to act as valves. They may be 'on', in the green condition, permitting the passage of fluids through them, or they may be permanently 'off', after drying, preventing fluid flow. In practice, however, the situation is not so simple. Debris may become trapped between the torus and the pit border, or the surface of the torus and pit border may be sufficiently rough to prevent perfect sealing (Stone 1939). Some aspirated pits are therefore capable of passing some fluids (Thomas and

Nicholas 1966, Comstock and Cote 1968, Meyer 1971).

Pit aspiration occurs not only when wood is seasoned but also in the standing tree as the sapwood moisture content falls during heartwood formation (Phillips 1933). Typically green heartwood is found to be less permeable than green sapwood from the same log and the difference is attributed to the aspiration of bordered pits. Krahmer and Cote (1963) suggested that heartwood pits are occluded with extractives and encrusted with lignin-like substances and these also contribute to heartwood's poor permeability. Heartwood permeability is generally unaffected by seasoning, although in some cases the permeability has been shown to improve marginally (Erickson and Crawford 1959). As heartwood is intrinsically less permeable, such observations are of little practical value to wood preservation.

Several investigators have specifically examined the flow of preservatives into softwoods. These include Wardrop and Davies (1961), Krahmer and Cote (1963), Erickson and Balatinecz (1964) and Bailey (1965) to name a few. The unanimous conclusion from these studies is that most preservative penetration into seasoned timber occurs from tracheid to tracheid via the bordered pits. Thus while it can be shown that seasoning causes pits to aspirate with a concomitant loss in permeability, sufficient numbers of bordered pits remain partially open, so allowing intercellular movement of fluids. Even though such a simple cause and effect hypothesis has been supported by many experimental observations, some anomalies have been noted which imply that permeability is not perfectly correlated with bordered pit aspiration (Sargent 1960). These anomalies

have led investigators to consider the relative effectiveness of other flow paths, such as the rays and the supposed transient cell wall capillaries, in permitting the flow of preservative into seasoned timber (Sargent 1960, Bailey 1965). Rays have been shown to function effectively in some timbers, mainly in localised redistribution of oil-borne preservatives from the tracheids. While the presence of cell wall capillaries has been revealed in electron microscope sections of a number of woods using special preparatory techniques, it is doubtful that their influence on preservative flow is significant compared to the flow through bordered pits, purely on the basis that the capillaries are so small (Stamm 1963). Some workers have argued that capillary size, as seen in electron microscope sections, does not reflect the true situation of wood under considerable treatment pressure when the capillaries may be expected to dilate (Bailey 1966).

2.4 PHYSICAL ASPECTS OF PRESSURE IMPREGNATION

The rate of fluid flow through wood can be estimated using equation 2-2 - an adaptation of Poiseuille's Law (Stamm 1946, 1964). The equation applies under only very simplified conditions:

[1] wood is taken to be a bundle of 'N' capillary tubes each of constant length and diameter connected in parallel

[2] the capillaries are completely filled with fluid

$$V = N \times \Pi \times R^4 \times P / 8 \times U \times L \qquad (2\text{-}2)$$

where V = rate of flow ($m^3 s^{-1}$)
P = pressure (Nm^{-2})
L = length of the capillary (m)
U = viscosity of the fluid ($10^{-1} Nm^{-2}.s$)
R = radius of a capillary (m)

Reality is unfortunately quite different: wood is far from being a bundle of regularly shaped capillaries and usually only dry wood is preservative treated. However, equation 2-2 serves to illustrate that the rate of flow is dependent on the fourth power of the capillary radius. When capillaries are arranged in parallel, the rate of flow is determined by the width of the largest capillary. The situation is more complex in wood, because the cell lumina, pit cavities and pit membranes can all be considered as capillaries in series, as well as in parallel. The rate of flow through such a series path will have to be equal in every capillary, but the pressure drop through the smallest capillary must of necessity be higher than that through the much larger cell lumen. Hence it can be seen that the size of pit membrane pores determines the rate of flow in softwoods. The number and size of the pit membrane pores in series through which a fluid must pass thus determine permeability. Longitudinal flow is greater than transverse flow because, per unit distance, fluids must cross 1/50 to 1/100 the number of pits than in a transverse direction.

The first stage of a commercial, full cell preservative treatment requires the removal of air from the wood.

Removal of air achieves two things:

[1] it avoids the problem of compressing the trapped air by the incoming preservative and subsequent blowback following the release of pressure (this is a desirable feature of empty cell treatments)

[2] it reduces the extent of air-liquid meniscus formation which can markedly retard the flow of fluids through fine pores.

However Petty and Preston (1969(a)) demonstrated the difficulties involved in removing air from wood. Their results indicate that air-liquid menisci will be present during a commercial treatment, reducing the flow of preservatives into timber. Air-liquid menisci are formed during preservative treatment as preservative vapour, ahead of the moving liquid front, condenses in the fine pit pores. This prevents the escape of air from the fibre cavities and causes the poor permeability (Stamm 1963). Capillary condensation can occur in the fine openings at relative vapour pressures as low as 90 % but condensation occurs in only the larger openings at saturation levels (Stamm 1964). High external pressures are needed to displace the air trapped by condensed vapour. The Laplace formula relating the displacement pressure to the size of the pores is given in equation 2-3.

$$P = 2\,(\sigma) / R \qquad (2\text{-}3)$$

Where P = pressure (Nm^{-2})
σ = surface tension (Nm^{-1})
R = maximum effective pit pore radius (m)

When 'R' is small the pressure required to displace trapped gas is high; when 'R' is large the opposite is

true. Equation 2-3 indicates that a tracheid must have a minimum pore radius of 0.1 μm before the highest pressures used in conventional treatment cylinders (approximately 1.4 M Nm^{-2}) overcome capillary tension and fill the cell lumen with water-based preservative. Stamm (1970) found that the maximum pore radius of most heartwoods is less than 0.1 μm so that effective treatment requires higher than normal pressures. Indeed, Blew (1961) showed empirically that it is possible to increase the uptake of creosote in coastal Douglas fir by increasing the treatment pressure. While an increase in pressure may solve the permeability problem extensive collapse of the tracheids occurs eventually and the technique has limited uses. Stamm (1970) demonstrated that lowering the surface tension of fluids with surfactants increased fluid uptake at a given pressure. An alternative approach to circumvent these difficulties is to increase the size of the limiting pit pores in softwood; that essentially is the subject of this thesis.

To summarise, the rate of preservative flow into wood is almost entirely determined by the size of the fine pores in the pit membranes. Sufficiently high pressures must be used to overcome the effect of air-liquid menisci and to displace trapped air.

2.5 THE PERMEABILITY AND PRESERVATIVE TREATABILITY OF DOUGLAS FIR

The preservative treatability and permeability of Douglas fir have stimulated considerable research over many decades. Much of the published literature is of North

American origin and is almost entirely devoted to the treatability of Douglas fir heartwood by creosote and oil-borne preservatives. Less has been written on sapwood treatability, particularly with respect to water-borne preservatives; that subject is paramount to this thesis.

Douglas fir is indigenous to the Western North America and natural stands can be found from California to Washington and also in British Columbia, Canada. Differences in density, growth rates and treatability can be correlated with the growing region. It is possible to subdivide the species into two taxa, Pseudotsuga menziesii (Mirb.) Franco, which occurs on the coast and Pseudotsuga menziesii var. glauca (Mayr.) Franco, which occurs in the montane regions (Blew 1961). Several studies indicate that the permeability of Douglas fir is influenced by its geographic location (Miller 1961, Erickson and Estep 1962, Miller and Graham 1963). A classification system based on heartwood permeability was formulated by Maclean (1960) in which coastal Douglas fir is regarded as moderately difficult to penetrate and Rocky Mountain or interior Douglas fir as exceedingly difficult to penetrate. Unfortunately the separation into two classes is not perfect, because the same range of permeabilities also exists within coastal Douglas fir (Nicholas and Siau 1973).

Several investigations have attempted to correlate the impermeability of interior Douglas fir heartwood to wood anatomy (Griffin 1919, Phillips 1933, Krahmer 1961, Erickson and Balatinecz 1964, Bailey 1965, Liese and Bauch 1967, Bailey and Preston 1969 and 1970, Meyer 1971). Despite considerable effort, a satisfactory explanation has not been

found. The permeability of both coastal and interior Douglas fir is low, but the permeability of interior Douglas fir is lower. Aspiration of bordered pits and encrustation with extractives in never-dried heartwood accounts for the poor permeability observed in both, but it does not explain the difference in permeability between the two. Krahmer (1961) found that refractory (impermeable) Douglas fir tended to have shorter tracheids and smaller cell lumina than permeable Douglas fir. Meyer (1971) corroborated that work and in addition found that there were more pits/tracheid and more pits/unit volume of wood in the coastal variety.

There is general agreement that creosote uptake in Douglas fir sapwood is higher than that of heartwood (Erickson and Balatinecz 1964, Bailey 1965, Bailey and Preston 1969, Liese and Bauch 1967). Both sapwood and heartwood are considered refractory to water-borne preservatives (Carter 1975 unpublished, Liese et al. 1982). Tschernitz (1973) noted that the sapwood treatability by creosote in Rocky Mountain Douglas fir is extremely variable. Meyer (1971) suggested that differences in sapwood permeability could be partially explained by the degree of pit aspiration. In a comparison of never-dried coastal and interior Douglas fir he found that 32% of the pits were aspirated in interior specimens, but only 2% in coastal specimens. He noted also that there are more pits in each tracheid in coastal Douglas fir and concluded that at a given level of pit aspiration, the number of functioning pits in coastal Douglas fir is higher than in interior Douglas fir. The smaller the number of aspirated pits, the more permeable the wood.

Preservative flow paths in Douglas fir are generally similar to those previously discussed in Section 2.3. The bulk of longitudinal flow is from tracheid to tracheid through the bordered pit pairs. Latewood is more permeable than earlywood after drying because of the the resistance of latewood pits to aspiration. Bauch et al. (1970) found that both heartwood and sapwood bordered pits were encrusted with ultraviolet light absorbing materials (i.e. phenolic substances).

Estimates put the ratio of tangential to radial flow from 1:10 (Bailey 1965) to 1:14 (Erickson and Estep 1962). Tangential flow is much less than axial flow because of the number of cell walls which must be traversed per unit distance in a tangential direction. In the tangential and radial directions Bailey (1966) demonstrated that plots of flow rate against pressure are non-linear. He stated that this was hard to explain if bordered pits were the only factor limiting flow and concluded that other structures are likely to be involved. A likely candidate for that role is the cell wall capillary system. Preston (1959) considered the potential for transient cell wall capillaries to contribute to radial flow, a point taken up later by Bailey and Preston (1969 and 1970). It was suggested the poor permeability in heartwood could be explained by the fact that cell wall capillaries are occluded with extractives (Bailey and Preston 1970). However the small size of those capillaries was thought to preclude their involvement on a large scale.

There is disagreement as to the most effective radial pathways for preservative uptake. It is generally believed

that ray parenchyma are ineffective because of the presence of large amounts of cytoplasmic debris remaining in the cells and also because of the structure of the simple pits in parenchyma (Bailey 1966, Liese and Bauch 1967). Erickson and Balatinecz (1964) were able to demonstrate the movement of polystyrene from tracheids to ray parenchyma via the half bordered pits, but there was no movement from penetrated cells into adjacent parenchyma cells. They found that the most important pathways for radial movement are the ray tracheids. This is perhaps the logical choice because of the obvious similarity to axial tracheids. However, ray tracheid cells are much shorter and narrower than their axial counterparts. For a fluid to move a certain unit distance via the ray tracheids it must cross over a greater number of cell walls, consequently radial flow is less than axial flow (Erickson and Balatinecz 1964). Liese and Bauch (1967) found that the ratio of the area occupied by ray parenchyma to the area occupied by ray tracheids in Douglas fir was approximately 8:1. In contrast, in a permeable softwood such as Scots pine, that same ratio was 1:1. Because the total area occupied by ray tissues in the two species is similar, obviously the occurrence of ray tracheids in Douglas fir is lower than in Scots pine. From a theoretical and empirical stand point, Liese and Bauch were able to demonstrate a close relationship of ray tracheid area to radial permeability. Unhampered fluid movement through ray tracheids depends on the bordered pits' being free of encrustations. Bailey and Preston (1969 and 1970) found that the bordered pits between vertical tracheids in sapwood are typically unencrusted, but both the

sapwood and heartwood ray tracheid bordered pits are encrusted. It appears that this factor can be quite variable in Douglas fir (Bailey 1966, Liese and Bauch 1967). There is limited evidence to suggest that intercellular spaces between the ray cells could contribute to radial flow (Erickson and Balatinecz 1964, Liese and Bauch 1967, Bailey 1966), but this has not been conclusively proved.

CHAPTER 3

PRELIMINARY INVESTIGATIONS

3.1 BACTERIA IN WOOD:- A REVIEW

The complex interactions of bacteria and wood have been comprehensively reviewed by a number of authors (Liese 1970, Greaves 1971(a) , Liese and Greaves 1975, Rossell et al. 1973, Smith 1975). There is considerable confusion and contradictory information in the literature. Comparisons among different studies are difficult because different environments and organisms are involved. Decay is sometimes attributed to bacterial attack on the premise that 'the damage present could not be due to fungal attack'. Frequently bacteria are observed in association with fungi, particularly soft rot fungi and consequently it is difficult to separate the effects of one organism from another. Few studies have attempted to fulfil Koch's postulates by using the organisms isolated from degraded wood to reproduce the same degradation patterns in uninfected wood.

It is generally believed that colonisation and attack by bacteria is a slow process (Greaves 1969, Liese 1970, Rossell et al. 1973). That is not always true; for example, Greaves (1969) observed destruction of tracheid and ray cells in Pinus radiata after only three years exposure to bacteria. Furthermore, recent work has associated tunnelling and cavitation bacteria with premature failure (within 3-4 years) of P. radiata horticultural posts in New Zealand

(Nilsson and Singh (1984), Nilsson and Daniel (1983) and Daniel and Nilsson (1985)). Clearly, it is difficult to make generalisations about the rate of bacterial decay.

In general, wood must have a high moisture content, over a long period of time, before bacteria will colonise and attack the wood substance. Such situations occur when wood is stored in ponds, under sprinklers, buried in soil or submerged in the sea i.e. water-logged. Water saturation of wood excludes decay fungi and provides colonising bacteria with a competitive advantage. The presence of free water also facilitates movement of bacteria in wood and allows the diffusion of nutrients and waste metabolites to and from active bacterial cells.

Greaves (1971 (a)) subdivided the various types of bacteria associated with wood into 4 major categories:-

> [1] Those bacteria which affect the permeability of the wood but do not significantly alter its strength.
>
> [2] Those which are able to attack the cell walls, altering the strength of the wood.
>
> [3] Those which function as an integral part of the total microflora of wood and contribute towards its ultimate breakdown.
>
> [4] Those more passive colonisers which may be able to influence the organisms capable of causing breakdown through the production of inhibitory compounds.

Bacteria which affect permeability (category [1]) are most relevant to this thesis but bacteria which are capable of attacking the cell walls are also important. Extensive attack of cell walls is associated with long term exposure of wood to bacteria. Changes in permeability occur much

sooner.

There are a number of ways in which bacteria reportedly improve the permeability of wood. In the initial stages of colonisation bacteria derive most of their nutrient requirements from the tertiary lamella and also from the cytoplasmic contents of parenchyma cells (Greaves 1969, 1970(b)). The presence of encrusting material on the cell wall surfaces (the tertiary lamella) and on the bordered pit margos and tori is thought to reduce permeability. Removal of the hydrophobic tertiary lamella by bacteria renders the walls more permeable (Greaves and Foster 1970). Bordered pit chambers are colonised early by bacteria and the bordered pit margo encrustations may be removed improving permeability (Greaves 1971(a)).

The ray cells constitute the most concentrated supply of readily available nutrients to the bacteria and they are generally found to be invaded early in the colonisation process. Extensive colonisation quickly depletes the ray cell contents. In certain situations, once the ray contents have been depleted degradation of the cell wall material can occur. Greaves (1969) recognised two patterns for this type of degradation:

> [1] non-destructive attack at the ultrastructural level affecting the cellulose microfibrils and causing a change in the birefringence.
>
> [2] a more destructive process in which the ray cell walls are completely removed.

He suggested that both forms of attack would improve permeability. Complete destruction of ray parenchyma has

been associated with large increases in permeability (Ellwood and Ecklund 1959, Knuth and McCoy 1962).

Attacks on the bordered pits are manifest in a number of different forms. Greaves (1969) was able to identify three distinct forms of attack which he called type 1, type 2 and type 3. He suggested that all three would lead to increased permeability. Type 1 attack involves random lysis inside and outside the pit chamber and results in discrete holes in the pit border. In extreme cases the border can become detached. Type 2 attack occurs infrequently and only in aspirated pits. Bacteria accumulate around sealed pit apertures damaging the pit border close to the aperture. Type 3 attack occurs from inside the pit chamber and is typified by a 'lacy' appearance to the pit border as it is decayed around the annulus.

Preferential attack of the bordered pit membranes is commonly observed during water storage. In some cases the margo can be readily degraded by bacterial cellulases causing the torus to be dislodged, preventing pit aspiration and consequently improving permeability. Degradation of the pectin-rich torus is also observed with the same effect on permeability. According to Liese (1970) bacterial attack of the pit membranes during water storage is limited to the sapwood. Lignification of bordered pit tori occurs during heartwood formation hindering the action of pectinases and cellulases. Not all sapwood bordered pits are equally amenable to enzyme attack (Bauch et al. 1970). Nicholas and Thomas (1968) demonstrated the difficulty of degrading the bordered pit tori of Douglas fir sapwood with commercial enzyme preparations. Bauch et al. (1968) reported that the

pit tori in Douglas fir sapwood contained appreciable quantities of phenolic materials.

Greaves (1969) stated that all cell types in wood can be colonised by bacteria and that all are equally susceptible to attack regardless of the degree of lignification. Greaves (1970(b)) supported that by demonstrating the degradation of P. radiata heartwood by selected bacteria. However, Greaves (1973) observed that the sapwood of Eucalyptus regnans was more readily degraded than the heartwood of radiata pine; he attributed that to differences in lignification. Courtois (1966) also found that all cell types in a number of softwood species examined were susceptible to microbial enzyme attack. However, he noted differences in the relative ease of degradation between early wood and late wood which he attributed to differences in lignification. The middle lamella region also resisted attack. In contrast, Boutelje and Bravery (1968) found that the latewood tracheids in Scots pine building piles were most susceptible to attack. Thus it appears that given time all components of wood will be attacked by bacteria. The apparent contradictions in the literature undoubtedly relate to different stages of decay and to different environmental conditions.

After initial colonisation bacteria migrate deeper into the wood. The rate of migration, method of movement and pathways used to travel from cell to cell are poorly understood. There have been few attempts to quantify the process. Greaves (1971(a)) speculated on the possibility that bacteria moved by cell division along cell surfaces. Using calculations based on the size of bacteria

and cell replication times he calculated that in P. radiata, ten tracheid widths or ten parenchyma lengths could be traversed in a 24 hour period. Greaves assumed that the pits and cell walls were penetrable.

Many bacteria found in association with wood are flagellated and are theoretically capable of limited movement. In one study Greaves (1973) found that motility did not confer any advantage to bacteria in promoting wood decay. Both motile and non-motile bacteria produced similar strength losses in Eucalyptus regnans but Greaves conceded that the similar result could have been a reflection of the small size of the inoculated specimens (less than 2.5mm wide). Non-motile bacteria could conceiveably be carried from cell to cell in water currents (Greaves 1970(b)).

It is generally assumed that bacteria pass from cell to cell via the pits (Harmsen and Nissen 1965, Boutelje and Bravery 1968, Greaves 1970(b)). That does not seem feasible unless the pits are damaged in some way. Greaves (1971(a)) suggested that loss or damage to the margo fibrils in bordered pits was necessary before bacteria could pass from tracheid to tracheid. In this context, experiments were attempted at Imperial College, London to impregnate Scots pine and Sitka spruce logs with a bacterial suspension using vacuum sap displacement (Newton 1979, unpublished). Results indicated that with Scots pine in particular, bacterial cells did not travel the whole length of the logs but were trapped at the butt-end by pit membranes. Greaves (1965) stated that bacteria were able to move from ray cells to tracheids in Scots pine even though there was no microscopic evidence of pit damage. He speculated on the existence of

alternative pathways through the cell wall.

A variety of common soil micro-organisms have been found in wood. Concise lists have been collated by Rossell et al. (1973) and Schmidt and Dietrichs (1976). The lists include the genera Pseudomonas, Flavobacterium, Serratia and most notably Bacillus. Bacillus polymyxa in particular is frequently isolated. More recently Cytophaga johnsonii (Kurowski and Dunleavy 1976(a)), Bacillus subtilis (Kurowski and Dunleavy 1976(b)) and Enterobacter cloacae (Macken and Pickaver 1979) have been implicated as being capable of improving permeability.

3.2 INITIAL TRIALS

Experiments were set up to devise a storage system capable of holding logs in a closed environment for several weeks. During that period it was intended to expose them to bacteria in an attempt to improve permeability. While under storage, the logs were to be sprinkled continuously with a nutrient salts solution in which bacteria were growing. A suitable bacterial inoculum and nutrient medium were therefore fundamental requirements of the system.

3.2.1 Isolation of suitable bacteria

Rather than using culture(s) of commercially available bacteria, organisms were isolated from water-stored logs and a screening program was conducted to isolate those which were likely to be most effective. The rationale behind such a selection procedure was simply that organisms isolated

from one water-storage environment might be expected to be more competitive than other organisms when returned to a similar environment.

Small wood samples were removed from Douglas fir logs which had been stored under continuous water sprinklers for 18 months. Three sampling techniques were employed:-

[1] a sterile increment corer

[2] saw dust collected in a sterile petri dish during chainsaw cutting

[3] small pieces cut from sawn discs using a sterile axe blade

Portions of each sample were homogenised with a high speed blender (ULTRATURRAX) for 60 seconds in 50 ml of sterile distilled water. One millilitre of the fibrous slurry which resulted was pipetted into a sterile petri dish and over-poured with 10 ml of nutrient agar (DIFCO). The plates were inverted, incubated at 25°C for seven days and examined for evidence of bacterial growth. Few bacterial colonies were observed and in many cases the isolation plates were over run with fungal growth. Fungi grew most heavily on plates inoculated with wood sampled using techniques [2] and [3] above. Those two techniques were the least sterile and it is possible that the fungi isolated were contaminants. The fact that bacteria were not isolated was attributed to the length of time the logs had been stored and to successional changes in the microbial population.

Therefore another log pile was set up using 33 year old suppressed Douglas fir poles obtained from the Selwyn

plantation board in Canterbury. Three days after felling, ten poles with intact bark were placed under a continuous water spray. At the commencement of spraying (February 1982), samples were removed using a sterile corer and plated onto nutrient agar as described previously. This was to assess the bacterial flora naturally resident in freshly felled logs. No bacteria were isolated. The sampling procedure was repeated after three weeks sprinkling; this time large numbers of bacteria were isolated.

Discrete colonies appearing on the isolation plates were sub-cultured onto fresh nutrient agar. In this way 12 isolates were obtained and set aside for further investigation. Discrete colonies were assumed to represent single species of bacteria.

3.2.2 Screening trials

A screening procedure was implemented to assess the capability of each of the isolated organisms to improve permeability. The following parameters were considered important:-

[1] enzyme production

[2] the ability to degrade pit membranes

[3] the ability to utilise wood as the sole energy source

Individual isolates were assessed accordingly after inoculating each organism into sterile flasks containing small (1.5cm), green cubes of Douglas fir sapwood, partly immersed in diluted nutrient broth (DIFCO). The cubes had

been sterilised earlier with propylene oxide for seven days. Once inoculated the cubes were incubated for three weeks at 25°C on a rotary shaker. Changes in permeability after the incubation period were measured using a simple dip test in a non-polar solvent, n-Hexane and also by mild pressure impregnation with aqueous 1% (w/v) aniline blue dye. The dip technique involved oven drying the cubes, weighing them, dipping them in n-Hexane for 12 seconds, air drying for 15 seconds and then reweighing. Any increase in weight uptake of the solvent above non-inoculated cubes was taken as evidence that the permeability had increased (Greaves 1970 (b)). The staining of the wood by the aniline blue dye allowed comparisons among depths of penetration. The ability of the organisms to alter pit membrane structure was investigated by incubating 20μm thick sections of sapwood with each organism for 48h. Sections were examined for evidence of pit damage using a Cambridge stereoscan 250 scanning electron microscope.

The importance of pectinase enzymes in the destruction of pit membranes has already been discussed in Chapter One. All bacterial isolates were screened for pectinase production using solid agar media (Hankin et al. 1971) as detailed in Appendix M6. While high pectinase activity is a distinct advantage in an isolate, cellulase activity should be minimal. Cellulase enzymes have the potential to attack the cell wall structure and in so doing affect the strength of the wood detrimentally. Bacteria were therefore screened for carboxy-methylcellulase activity (Saddler 1982).

After conducting the trials one culture from the original 12 isolations proved superior to the others in

every respect and was selected for further analysis.

3.2.3 Bacterial Identification

Examination of a gram stained smear from the selected culture revealed that it was not a single bacterial species but a mixed population. Further streaking on to fresh nutrient agar separated at least six different organisms, distinguishable on the basis of colony pigment, cell shape and gram staining characteristics. A range of physical and biochemical tests were undertaken to tentatively identify the organisms. The tests included measurements of cell size, cell shape, motility, heat survival, catalase activity, acid fastness, anaerobic growth, starch utilisation, and the Vosges Proskauer test. The techniques involved are outlined in Appendix M7 and the results tabulated in Table 3-1. Using the scheme of Degroot and Johnson (1976) presumptive identifications were made. Five of the six cultures were either gram positive or gram variable rods. Two of those cultures, designated 1 and 4, survived immersion in a boiling water bath for 10 minutes suggesting that they were spore formers. Spore formation on nutrient agar media was rare even in 3-4 week old cultures but on tryptic soy media enriched with Mn++ ions, spores were formed readily after incubation at 30 °C for four days. An attempt was made to refine the identification of cultures 1 and 4 using the tests for *Bacillus* spp. described by Smith *et al*. (1952) and Bergey's Manual (Buchanan and Gibbons 1975). Spore size and shape, acid and gas production from a variety of sugar substrates, production of dihydroxyacetone from glycerol and

TABLE 3-1 RESULTS FROM PRELIMINARY BACTERIAL IDENTIFICATION PROCEDURES

ORGANISM CODE	GRAM STAIN	ANAEROBIC GROWTH	FERMENTATIVE GROWTH	SHAPE	MOTILITY	CATALASE ACTIVITY	ACID FASTNESS	HEAT SURVIVAL	STARCH UTILISATION	VOSGES PROSKAUER
1	VARIABLE	+VE	+VE	RODS	+VE	+VE	-VE	+VE	+VE	-VE
2C	VARIABLE	+VE	+VE	RODS	+VE	+VE	-VE	-VE	+VE	+VE
2W	VARIABLE	+VE	+VE	RODS	+VE	+VE	-VE	-VE	+VE	+VE
3	VARIABLE	+VE	+VE	RODS	+VE	+VE	-VE	+VE	+VE	+VE
4	+VE	+VE	+VE	RODS	+VE	+VE	-VE	+VE	+VE	S
5	-VE	+VE	+VE	RODS	+VE	+VE	-VE	-VE	+VE	+VE

S - INDICATES A SLIGHTLY POSITIVE REACTION

TABLE 3-2 ACID AND GAS PRODUCTION FROM A RANGE OF SUGARS UNDER OXIDATIVE AND FERMENTATIVE ENVIRONMENTS

CODE	GLUCOSE		XYLOSE		ARABINOSE		MANNITOL	
	OX	FER	OX	FER	OX	FER	OX	FER
1	+VE	+VE(G)	-VE	S(G)	+VE	+VE(G)	+VE	+VE(G)
2	+VE	+VE(G)	S	-VE	S	S	+VE	+VE(G)
3	+VE	+VE(G)	S	-VE	+VE	+VE(G)	+VE	+VE(G)
4	+VE	+VE(G)	S	-VE	+VE	+VE(G)	+VE	+VE(G)
5	+VE	+VE(G)	-VE	S(G)	-VE	+VE(G)	-VE	+VE(G)
6	+VE(G)	+VE(G)	+VE(G)	+VE(G)	-VE	-VE	+VE(G)	-VE

S ------ INDICATES SLIGHTLY POSITIVE REACTION
G ------ INDICATES THE PRODUCTION OF GAS

hydrolysis of casein were examined. The results are tabulated in Tables 3-1 and 3-2. On the basis of the accumulated data, cultures 1 and 4 are almost certainly Bacillus spp. and probably Bacillus polymyxa (Prazmowski) Mace.

Cells from cultures 2C, 2W and 3 were pleomorphic. A mixture of short rods and long curved rods with some branching was common in young cultures (2-3 days); as they aged, the rods became shorter and more regularly shaped. Young cultures were generally gram positive but that property was rapidly lost as the cultures aged. Irregular staining gave the cytoplasm a granular appearance. Their pleomorphic and gram staining properties suggest that they are coryneforms but no further testing was carried out to confirm this because of the difficulties associated with identifying this group (Buchanan and Gibbons 1975). None of the biochemical tests performed on these cultures supported their earlier separation based on cultural characteristics. It is possible that cultures 2C, 2W and 3 are all the same organism.

Culture 5 was conclusively gram negative and produced copious amounts of acid and gas from the fermentable sugars supplied as substrates. On the basis of the available data, culture 5 was identified as a member of the Enterobacteriaceae and is most likely to be an Enterobacter sp.

Trials were conducted using the species identified above, individually and in several combinations to determine which of the organisms present in the original isolation were most important. The trials were not comprehensive but

early indications of bacterial growth on defined media containing polygalacturonic acid (SIGMA) or pectin (SIGMA) as the carbon source suggested that growth and pectinase production was optimal in a mixed culture. On the basis of that result it was decided to conduct further experimentation in-vivo and in-vitro using the mixed culture. The name BPCE (short for Bacillus polymyxa-Coryneform-Enterobacter) was coined for the mixed culture and will be used from now on.

3.2.4 Selection of sprinkling media

Having isolated a suitable bacterial culture it was necessary to devise a sprinkling medium capable of sustaining optimal bacterial growth and enzyme production. A literature search was initiated. Pectinases are known to affect permeability; therefore attention was confined to literature on the optimal production of those enzymes.

Fernando (1937) found that the principle factor controlling pectinase production by Bacillus subtilis and B. carotovora was the pH of the culture medium; good enzyme production occurred between pH 5.5 and pH 8.5. The extent of any pH drift was influenced by the ratio of carbon (C) to nitrogen (N) (C:N); high C:N ratios lowered the pH and reduced growth and enzyme production. In contrast low C:N ratios resulted in an increase in pH and vigorous enzyme production. If the media were sufficiently buffered to prevent extreme pH values then similar enzyme concentrations could be obtained with a wide range of C:N ratios.

Hsu and Vaughn (1969) found that the production of

polygalacturonate transeliminase (PGTE) by Aeromonas liquefaciens was stimulated if growth was restricted. They observed that enzyme production was up to 500 times greater in carbon limited cultures than in cultures where excess carbon substrate was present. With polygalacturonic acid as a carbon source enzyme production could be enhanced if the culture pH was buffered above pH 7 or alternatively if a chelating agent, ethylene diamine tetra-acetic acid (EDTA), was present in the culture medium. No stimulation of enzyme production was observed through alkaline buffering or the addition of EDTA when glucose replaced polygalacturonic acid as the carbon source. Hsu and Vaughn explained the contrasting behaviour on the basis of substrate availability. They observed that PGTE activity, distinct from PGTE production, was highest in the presence of divalent cations. To degrade polygalacturonic acid the PGTE produced by the bacteria requires divalent cations; both EDTA and an alkaline pH restrict the availability of such ions. A shortage of divalent cations lowers the rate of substrate degradation. The organism responds by producing greater quantities of enzyme. No stimulation of enzyme production is evident with glucose because consumption of the substrate is not mediated by PGTE and hence does not depend on the presence or absence of divalent cations. The small amount of enzyme produced with glucose presumably represents a basal level produced constitutively by the bacteria. Stimulation of PGTE activity by divalent cations, in particular by Ca++ ions, has been reported by several other authors (Starr and Moran 1962, Fuchs 1965, Nasuno and Starr 1967).

More recent work (Ward and Fogarty 1974, Kurowski and Dunleavy 1976a,b and Macken and Pickaver 1979) has concentrated specifically on the production of pectinases by bacteria isolated from water stored wood. Ward and Fogarty (1974) noted that polygalacturonate lyase (PGL) synthesis by B. subtilis and Flavobacterium pectinovorum was influenced by the carbon and nitrogen source used to supplement a basal mineral salts medium. PGL activity by B. subtilis occurred on a variety of carbohydrates whereas F. pectinovorum required pectin as an inducer. Ammonium sulphate was found to be the best source of nitrogen for both organisms. Enzyme production by B.subtilis below pH 5.3 was inhibited but enzyme production by F. pectinovorum appeared independent of pH.

Relating the results of the studies just mentioned directly to the formulation of a culture medium presents some difficulties. Mature wood has a high C:N ratio which ranges from 350-500:1 but it has been reported as high as 1250:1 in Sitka spruce (Cowling and Merrill 1966). Compared to the amount of carbon, the total nitrogen content of wood is low; typically in the order of 0.03-0.1% by weight (Merrill and Cowling 1965). Indeed, total nitrogen levels in Douglas fir have been reported as low as 0.051% by weight (Allison et al. 1963). A proportion of the total nitrogen is reportedly soluble, and is thought to originate from the soluble proteins and amino acids contained in living parenchyma (King et al. 1974); the majority of the nitrogen present is highly insoluble and unlikely to be available to invading micro-organisms without extensive wall degradation (Merrill and Cowling 1965). Soluble nitrogen in Scots pine

wood has been estimated at being 14% of the total nitrogen present (Baker et al. 1970).

Although the nitrogen level is low and it contributes to the high apparent C:N ratio, in practice much of the total carbon present is not readily available. The bulk of Douglas fir woody tissue is composed of cellulose (42.8% of the oven dry weight) and lignin (26.4% of the oven dry weight)(Panshin and de Zeeuw 1980, p.92). The remaining 30% is hemicellulose. Since the bacteria isolated from water stored wood do not produce measurable quantities of cellulase enzymes or lignases most of the carbon contained in wood is unavailable to them. Only 10% of the total hemicellulose fraction or 3% of the total oven dry weight of wood consists of arabinogalactan, xyloglucan and pectin, degradable by the bacteria. Most of that material occurs in the middle lamella (Timmell 1965, Panshin and de Zeeuw 1980, p.96) and is therefore inaccessible to bacterial enzymes present in the cell lumens. On the other hand, free sugars and starches can be expected to contribute to the available carbon.

Smith and Zavarin (1960) found that free carbohydrates (glucose, fructose, sucrose, raffinose) in Douglas fir comprised 0.32% of the dry weight. This value is only an average because levels of sugars and starches are known to fluctuate seasonally (Kreuger and Trappe 1967). Even though the amounts of free sugars are small their accessibility suggests that they may be important as an energy source in the early stages of bacterial colonisation.

In summary, although the theoretical C:N ratio in wood tissue is very high the restricted availability of carbon in

particular reduces the ratio to a level which is more optimal for enzyme production.

It has been established that pH effects bacterial growth and pectinase production. Bacteria inside wood are influenced by the pH of the wood tissue itself, the pH of the cell sap and by also the effects of bacterial metabolism on those pH values. The natural pH of Douglas fir heartwood has been reported as low as pH 3.45-4.15 (Gray 1958) and pH 3.3 (Fengel and Wegener 1984). Sandermann and Rothkamm (1959) quoting Trendelenburg and Scherte (1937) report that the pH of Douglas fir sapwood ranges from pH 5.76-5.83, much less acidic than the heartwood. However the pH of the cell sap is likely to be more important to bacterial metabolism than the pH of the wood. This is because bacteria are in intimate contact with the cell sap. No measurements of cell sap pH were found in the literature but the pH must be in equilibrium with the surrounding wood. Hence, cell sap pH is likely to be similar to that of sapwood and consequently sub-optimal for bacterial growth and pectinase production.

Thus there is potential to manipulate the pH within wood tissue through the use of buffers and by controlling the C:N ratio. To evaluate those possibilities, a series of in-vitro experiments were devised as described in the following section.

3.2.4.1 In-vitro experimentation

Following the work of Hsu and Vaughn (1969) and Fogarty and Ward (1973) a basal mineral salts medium was supplemented with two different concentrations of ammonium

sulphate as a nitrogen source and two different concentrations of phosphate buffer. Glucose at a growth limiting concentration of 5g/l was added as a carbon source. Details of the individual media used can be found in Table 3-3. Media were sterilised by autoclaving; glucose and

TABLE 3-3 COMPOSITION OF MEDIA USED IN EXPERIMENT 1

NUTRIENT	MEDIUM 1	2	3	4
K_2HPO_4	17	17	8.5	8.5
KH_2PO_4	4	4	2	2
NaCl	0.5	0.5	0.5	0.5
$MgSO_4$ (b)	0.5	0.5	0.5	0.5
$CaCl_2$ (a)	0.01	0.01	0.01	0.01
$FeCl_3$ (a)	0.001	0.001	0.001	0.001
GLUCOSE (b)	5	5	5	5
$(NH_4)_2SO_4$	0.5	5	5	5
pH INITIAL	7.50	7.37	7.37	7.24
C:N	20:1	2:1	20:1	2:1

ALL TABULATED VALUES REPRESENT CONCENTRATIONS IN g/l
a - dissolved separately
b - autoclaved separately and mixed before use

magnesium sulphate were autoclaved separately and mixed with the rest of the medium immediately before use. Two hundred millilitre quantities of each medium were inoculated with 1ml of a BPCE culture (grown in glucose peptone broth for 24h) and incubated at 30°C without shaking. At increasing time intervals samples were removed and analysed for bacterial growth, pH changes and enzyme activity. Growth was assessed by measurement of the optical density at 540nm in samples removed from each flask at different time

intervals. Non-inoculated medium was used as a blank. Optical density measurements were converted to oven dry weights of cells using a standard curve. Enzyme activity was measured by the change in absorbance at 235nm in a double beam spectrophotometer (SHIMADZU) (Appendix M5). Differences in enzyme production among the four media could be compared on an equivalent cell basis after standardisation. Results for all analyses are presented in Figure 3-1.

The initial inoculum contained too few bacteria. Once the inoculum was diluted into 200ml of medium there was a considerable time lag before a significant change in culture turbidity occurred. Bacterial growth was eventually highest in medium 4. The culture pH fell slightly in all media but the effect was greatest with the lower buffer concentrations in medium 3 and medium 4. The production of enzyme by the mixed culture was highest in medium 4 which had the lowest buffer concentration and the lowest C:N ratio. Interestingly, pectinase production was absent in medium 2 which had a high buffer concentration but a low C:N ratio suggesting that the buffer was in some way limiting enzyme production.

The experiment was repeated using glucose at a concentration of 10g/l to investigate the effect of carbon availability on enzyme production. To maintain the C:N ratio at a level similar that in the first experiment, the amount of nitrogen added was also increased. Details of the media composition for experiment 2 can be found in Table 3-4. Individual media were dispensed into 100ml quantities and inoculated with 1ml of a BPCE suspension grown in

TABLE 3-4 COMPOSITION OF MEDIA USED IN EXPERIMENT 2

NUTRIENT	MEDIUM 1	2	3	4
K_2HPO_4	17	17	8.5	8.5
KH_2PO_4	4	4	2	2
NaCl	0.5	0.5	0.5	0.5
$MgSO_4$ (a)	0.5	0.5	0.5	0.5
$CaCl_2$ (a)	0.01	0.01	0.01	0.01
$FeCl_3$ (a)	0.001	0.001	0.001	0.001
$MnSO_4$ (a)	0.0001	0.0001	0.0001	0.0001
GLUCOSE (b)	10	10	10	10
$(NH_4)_2SO_4$	10	5	10	5
pH INITIAL	7.21	7.28	7.03	7.13
C:N	2:1	4:1	2:1	4:1

ALL TABULATED VALUES ARE CONCENTRATIONS IN g/l
a - dissolved separately
b - autoclaved separately and mixed before use

glucose peptone broth for 72h rather than for 24h as in experiment 1. This produced a much higher inoculum concentration. Results for culture growth, pH drift and enzyme production are presented in Figure 3-2.

The graph shows clearly that bacterial growth is greater in the glucose enriched media. Growth is highest in medium 3. The pH drift was most severe in media 3 and 4 which had the lowest buffer concentrations. The graph of enzyme activity indicates that PGTE production was extremely poor in all media. Low buffer concentration and a low C:N ratio favoured enzyme production in experiment 1 but not in experiment 2. The major difference between the two experiments was the glucose concentration. The poor enzyme production in experiment 2 could have been due to the

greater availability of carbon. An alternative hypothesis was that PGTE production was not actually stimulated by the medium in experiment 1 and that the small amounts of enzyme detected were actually constitutive enzymes.

A third experiment was devised to investigate those hypotheses. Bacteria were inoculated into nutrient media containing a lower buffer concentration and a lower C:N ratio than that used in experiments 1 and 2. A third medium containing polygalacturonate as the carbon source was also set up. Details of the media composition can be found in Table 3-5. Results are presented in Figure 3-3.

TABLE 3-5 COMPOSITION OF MEDIA USED IN EXPERIMENT 3

NUTRIENT	MEDIUM 1	2	3
K_2HPO_4	8.5	4.25	8.5
KH_2PO_4	2	1	2
NaCl	0.5	0.5	0.5
$MgSO_4$ (b)	0.5	0.5	0.5
$CaCl_2$ (a)	0.01	0.01	0.01
$FeCl_3$ (a)	0.001	0.001	0.001
$MnSO_4$ (a)	0.0001	0.0001	0.0001
GLUCOSE (b)	2.5	2.5	0.0
POLYGALACT-URONATE (b)	0.0	0.0	2.5
$(NH_4)_2SO_4$	5	5	5
pH INITIAL	7.10	7.03	7.01
C:N RATIO	1:1	1:1	1:1

ALL TABULATED VALUES ARE CONCENTRATIONS IN g/l
a - dissolved separately
b - autoclaved separately and mixed before use

Bacterial growth was highest in medium 3 which contained polygalacturonate as the carbon source. Growth was lowest in medium 2 with the lowest concentration of

Figure 3-1 Bacterial growth, PGTE production and pH drift in artificial media Experiment 1

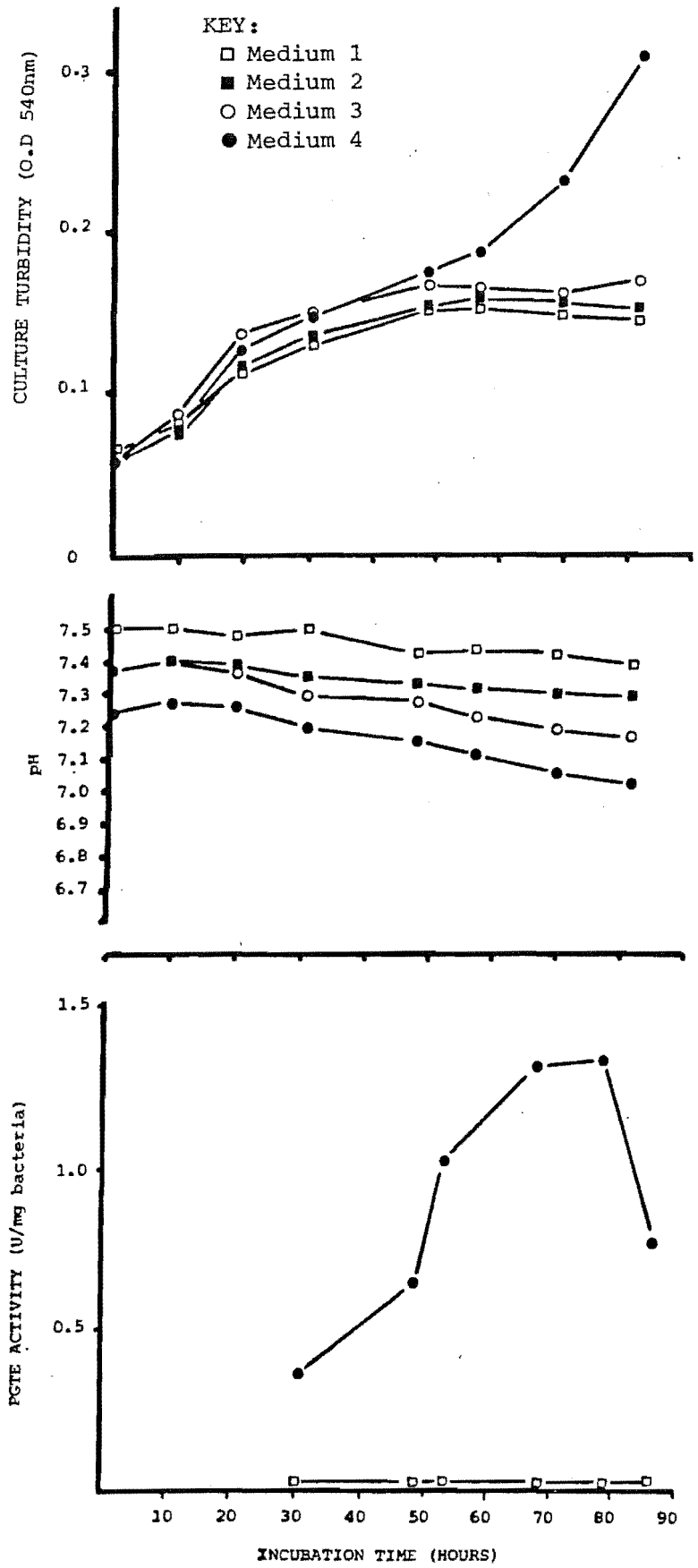
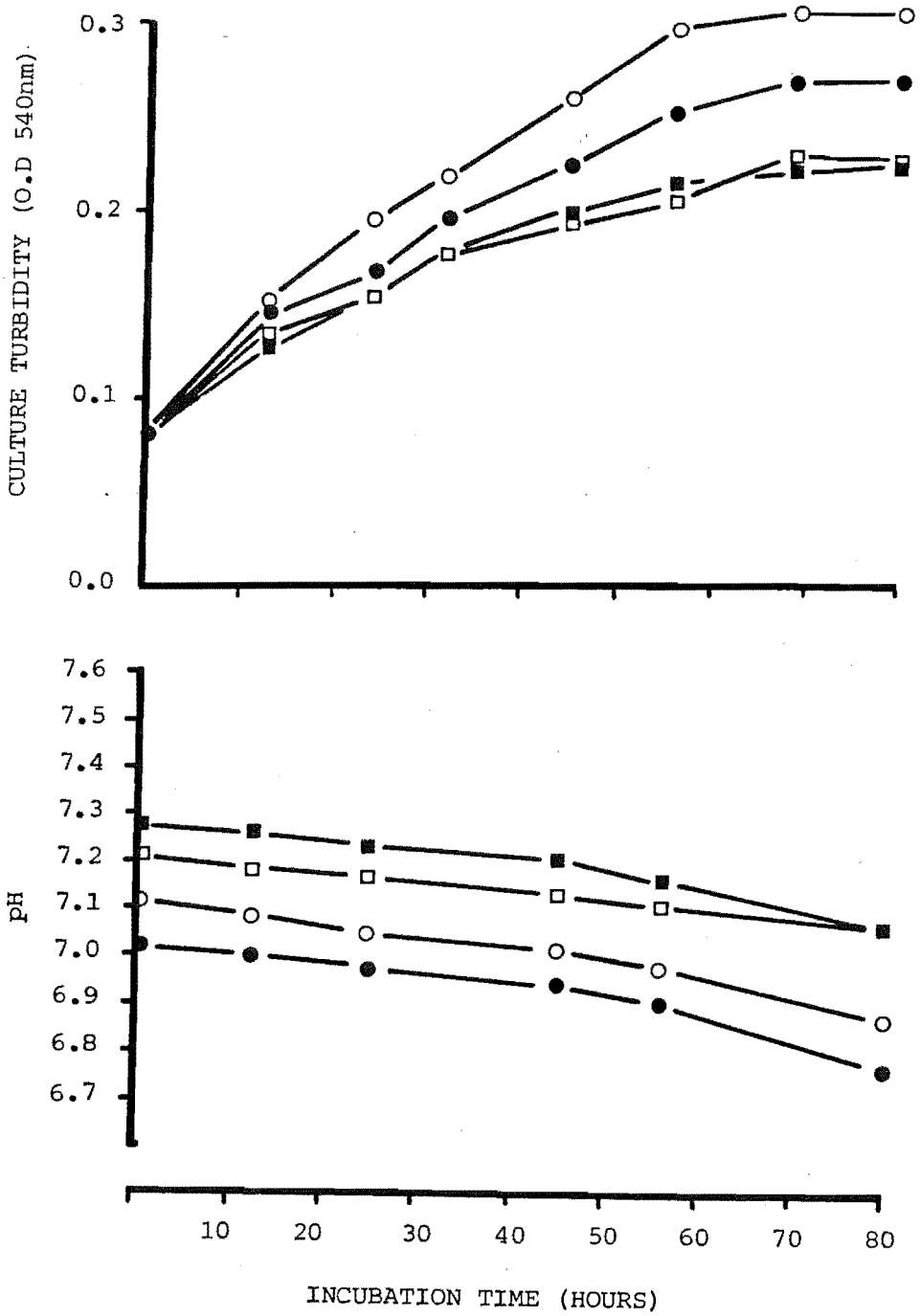


Figure 3-2 Bacterial growth, PGTE production and pH drift in artificial media Experiment 2

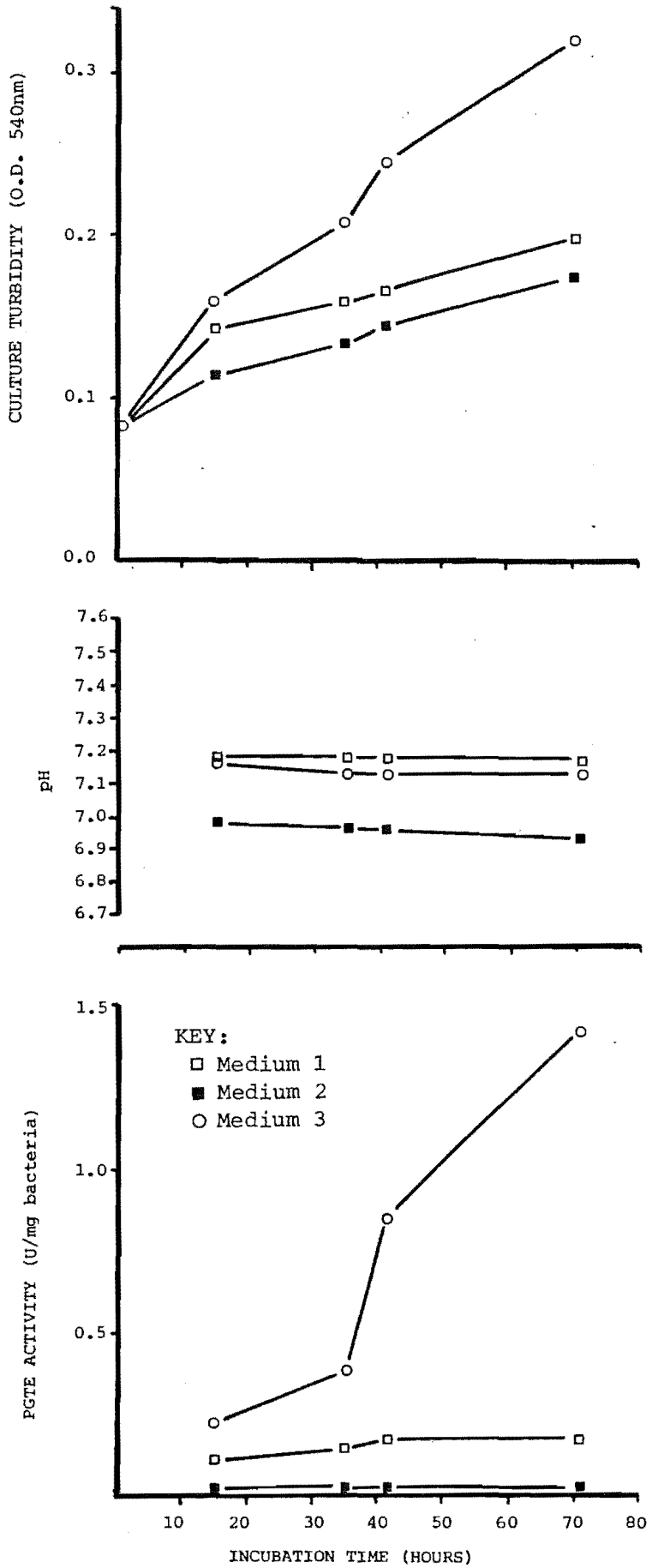


KEY TO SYMBOLS:

- □ Medium 1
- ■ Medium 2
- ○ Medium 3
- ● Medium 4

Note no significant PGTE activity was produced in any of the four media

Figure 3-3 Bacterial growth, PGTE production and pH drift in artificial media Experiment 3



buffer. Trace levels of PGTE activity were detected in the cell-free supernatant of medium 1. No activity was detected in medium 2 but significant levels of activity were assayed in medium 3. The results suggested that PGTE production by the mixed bacterial culture was in fact induced by polygalacturonate present in the medium.

3.2.4.2 In-vivo experimentation

The next step was to establish whether the conditions for bacterial growth and enzyme production found to be optimal in-vitro were equally effective in-vivo using wood as the sole carbon source. A comprehensive series of trials using small wood cubes and a range of different nutrient regimes was attempted. In practice this was a difficult and time consuming task. One of the greatest problems encountered was maintaining sterility. Sawn wood cubes are of course inherently non-sterile; sterility is essential to ensure that any changes occurring in the cubes after inoculation are not due to contamination. Sterilisation of media and wood by steam autoclaving was not considered a viable alternative because of the possibility that the wood cubes would be chemically and physically altered by excessive heat. Propylene oxide was used to sterilise cubes before they were added to an autoclaved nutrient solution. There were a number of recurring problems associated with propylene oxide sterilisation. In some cases it appeared ineffective; non-inoculated 'sterile' cubes contaminated sterile nutrient medium. In other instances, no growth occurred after inoculation presumably because sufficient

propylene oxide to kill the inoculum still remained in the cubes. A combination of contamination in some treatments and the absence of growth in others made conclusions on the effects of the C:N ratio and phosphate buffering of questionable value. Time did not permit a repeat of the whole experiment. However, repeating part of the experiment using a single, fixed nitrogen and buffer concentration provided some information about the sequence of bacterial colonisation and enzyme production. Experimental details and results are presented below.

Three green, sterile, 1.5cm cubes were added to each of twenty flasks containing 100ml of a nutrient salts solution (Table 3-6). Half of the flasks were inoculated with 1ml of a BPCE suspension grown in glucose peptone broth for 72h, the remainder were kept sterile to act as controls. All flasks were incubated at 25 °C with gentle agitation on a rotary shaker. At weekly intervals, one control and one inoculated flask was removed for analysis. The culture pH, concentration of reducing sugars and relative pectinase activity were measured as described in Appendix M8. Bacterial numbers in the culture fluid and also inside the cubes were estimated using a catalase enzyme assay (Line 1983) (Appendix M9). Results of all analyses during the 11 week incubation period are presented in Table 3-7.

The pH in both control and inoculated flasks fell slightly over the 11 week incubation period, indicating effective buffering. During the first two weeks, the concentration of reducing sugars present in the culture fluid was the same in both inoculated and control

TABLE 3-6 COMPOSITION OF THE BASAL MINERAL SALTS MEDIUM USED FOR IN-VIVO EXPERIMENTATION

NUTRIENT	AMOUNT (g/l)
KH_2PO_4	17
K_2HPO_4	4
NaCl	0.5
$(NH_4)_2SO_4$	5
$MgSO_4$	0.5
$MnSO_4$	0.001
$FeSO_4$	0.0001
INITIAL pH	7.25

TABLE 3-7 RESULTS AFTER INCUBATION OF DOUGLAS FIR CUBES IN MINERAL SALTS MEDIUM FOR 11 WEEKS

		INCUBATION TIME (WEEKS)							
		0	1	2	3	4	6	8	11
CULTURE pH	CONTROL	7.33	7.06	7.00	7.01	6.95	6.93	6.87	6.83
	INOCULATED	7.32	7.05	7.04	7.04	7.04	7.02	6.93	6.91
RELATIVE REDUCING SUGAR	CONTROL	0	0.164	0.182	0.210	0.191	0.130	0.130	0.130
	INOCULATED	0	0.145	0.180	0.380	0.380	0.280	0.250	0.220
CATALASE ACTIVITY IN CULTURE	CONTROL	0	0	0.43	0.37	0.62	0	0	0
	INOCULATED	1.2	4.4	3.9	2.5	3.2	3.0	0.94	1.45
CATALASE ACTIVITY IN CUBES	CONTROL	----	0.58	0.25	0	0.38	0	0	0
	INOCULATED	----	0.10	0.83	12.0	29.1	31.2	9.3	9.9
RELATIVE PECTINASE ACTIVITY	CONTROL	----	----	----	0	0.001	0.001	0.043	0.005
	INOCULATED	----	----	----	0.038	0.059	0.073	0.116	0.021

(----) INDICATES THAT NO MEASUREMENTS WERE TAKEN

flasks. After three weeks, the level in the inoculated flasks doubled. Presumably this reflected the liberation of reducing groups from wood polysaccharides as a result of bacterial metabolism. From the third week onwards, reducing sugar level in the inoculated flasks remained twice that in the controls.

Bacterial growth in the inoculated culture medium, as measured by catalase activity, peaked after one week and then showed a steady decline. There was little catalase activity in macerated wood cubes until the second week of incubation. Activity peaked at a level 7-8 times higher than the maximum occurring in the culture fluid, after six weeks incubation.

The thiobarbituric acid assay for pectinase activity was not found to be particularly sensitive and since there was no calibration against other techniques the results are suitable only for comparison with the control flasks. No activity was detected in the culture fluid until three weeks had elapsed. Activity peaked after eight weeks incubation. Pectinase activity inside the wood cubes was not measured.

Though the information provided by this experiment is limited it serves to show that the measurement of bacterial cell numbers outside of the wood cubes bears no relationship to population growth inside the wood. Hence, culture fluid pH is of minor importance since the majority of the bacteria are inside the wood and they are influenced only by the cell sap pH. It is unfortunate that pectinase activity was not measured inside the cubes since measurements outside the wood in the culture fluid are of questionable value.

3.2.4.3 Summary

The results from in-vitro studies reported in the literature, indicated the importance of the C:N ratio for optimal pectinase production. The few in-vitro experiments reported here failed to demonstrate such a relationship but it was discovered that pectinase production was induced by the presence of polygalacturonate in the medium. It has been established that the C:N ratio on a micro-scale relevant to bacteria within wood is much lower than that assumed from total carbon and nitrogen levels. Even so the maintenance of a low C:N ratio through the addition of exogenous nitrogen to logs under water-storage is likely to be of considerable advantage in maintaining optimal growth and pectinase production. Even though the advantages of added nitrogen and phosphate buffering were not actually observed in-vivo using wood cubes, it was decided to incorporate both chemicals into a medium for use in full scale sprinkling trials, solely on the basis of results from in-vitro experiments.

3.3 FULL SCALE SPRINKLING TRIALS

Four robust tanks, each measuring 1 X 0.85 X 0.5m were fabricated from plastic sheeting to provide containers capable of holding wood and liquid culture medium. A single outlet hole was drilled in the bottom of each tank and fitted with a 1.5cm diameter PVC pipe connection. Filters were attached to the pipe connectors inside the

tanks. Outside the tanks, small rotary pumps (EHEIM) were joined to the connectors by short lengths of polyethylene tubing. Spray bars, running the entire length of each tank, were attached to the outlet side of the pumps. Each pump had a capacity of 20 l/min to a head of 1m. By varying the number and position of the holes drilled in the spray bars, water spray could reach all areas of the tanks evenly. The arrangement of tanks, pumps and spray bars is shown in Figure 3-4.

Figure 3-4 Photograph showing the arrangement of sprinkling tanks, pumps and spray bars used in sprinkling experiments

The sprinkling system allowed the following aspects to be investigated:-

[1] The influence of nutrients (ammonium sulphate and phosphate buffer) on permeability changes

[2] The influence of mechanical incising in enhancing the rate of bacterial egress into the wood

[3] The desirability of a cyclic sprinkling regime

Timber for use in the trial experiment was felled from a 25 year old stand of Douglas fir at Ashley State Forest, Canterbury. Logs were stored in 1.8m lengths at 4°C, end sealed and wrapped in heavy polythene sleeving. When required for the sprinkling treatment, logs were debarked by hand, sawn into 0.5m lengths and then quartered. It was possible to cut 12 pieces from each log. Thirty six pieces originating from three logs were numbered and divided randomly into six groups of six pieces. Four of the six groups were incised to a depth of 2cm on the tangential face only. One incised and one non-incised group were set aside as non-sprinkled controls. The remaining four groups were placed on spruce heartwood bearers in separate sprinkling tanks. The bearers ensured that no part of the wood was submerged when the sprinkling solution was added. In effect, the six groups constituted six different treatments, summarised as follows:

[1] Incised, no sprinkling

[2] Incised, cyclic sprinkling, no nutrients

[3] Incised, cyclic sprinkling, nutrients

[4] Incised, constant sprinkling, nutrients

[5] Not incised, cyclic sprinkling, nutrients

[6] Not incised, no sprinkling

The nutrient solution consisted of the following:

K_2HPO_4	306 g
KH_2PO_4	72 g
$MgSO_4$	9 g
$(NH_4)_2SO_4$	90 g
NaCl	9 g
$FeSO_4$	0.01g
$CaCl_2$	1.8 g
pH	7.2

dissolved in 18 litres of distilled water. The sprinkling cycle involved two hours of sprinkling with one hour off. All four tanks were inoculated with 500ml of a BPCE suspension grown in diluted nutrient agar for three days. Tanks were set up on a laboratory bench with no temperature or humidity control. Ambient room temperature fluctuated diurnally from 11°C at night to 15°C during the day. Relative humidity often fell as low as 30% which meant that evaporation from the tanks was high and constant topping up with fresh distilled water was required. The experiment was terminated after eight weeks sprinkling and the samples were kiln dried in preparation for a standard Bethel preservative treatment using copper-chrome-arsenate. The results for this preliminary investigation can be found in Appendix P1 and formed the basis of a paper presented at the New Zealand Wood Preservers Annual Conference in 1983 (Archer 1983). To summarise those results, a comparison of the non-sprinkled with sprinkled bolts indicated quite conclusively that sprinkling improved preservative uptake by a factor of 3.5 in non-incised bolts and by a factor of two in the incised bolts. However, at its best, in treatment 4 (constant sprinkling, nutrients and incising), preservative uptake was

only 60% of that theoretically possible. There appeared to be no advantage in sprinkling with a nutrient solution and incising had no effect on preservative uptake after sprinkling. Incising did however, double the uptake of preservative in the non-sprinkled controls. It is surprising that the variability in treatment 3 (incised, cyclic sprinkling and nutrients) was much lower than that in other treatments.

The eight week sprinkling time had been arbitrarily selected and it was probable that the system was not optimal for bacterial growth although there was no assessment of bacterial growth during the experiment. Maximum air temperatures during the day were measured at 14-15°C; typically the temperature of logs inside the tanks was 1-2°C lower due to evaporative cooling. To investigate the hypothesis that temperature had been a limiting factor in the first trial, the experiment was repeated, this time at a higher temperature. The tanks were placed in a thermostatically controlled room at 23-24°C. Humidity was also controlled at 60% r.h. to reduce the rate of evaporation. The pumps were left running continuously in all tanks because results from the first experiment indicated that there was no obvious difference between a cyclic or constant sprinkling cycle on preservative uptake.

The results from the second experiment are compared with those from the first in Appendix P2 - a paper presented to the 16th New Zealand Biotechnology Conference in 1984 (Archer 1984). In brief, sprinkling at a higher temperature resulted in a rise in the preservative uptake to a level close to the theoretical maximum. All sprinkling treatments

achieved the same level of preservative uptake. Therefore it was not possible to measure the effect of the addition of nutrients or incising. The eight week sprinkling period was probably sufficient to achieve maximum uptake without assistance from nutrients or incising; hence it was not possible to differentiate between the various treatments. A third trial was designed to test the hypothesis that nutrients and incising would cause a decrease in the amount of time taken to achieve 100% theoretical preservative uptake.

For the third trial round bolts rather than quartered bolts were used. To accommodate the larger material it was necessary to increase the size of the plastic tanks and the capacity of the sprinkling systems. Four new tanks were fabricated and linked together in pairs with polyethylene tubing. Each pair of tanks was served by one pump. One hundred and twenty litres of a sprinkling medium identical to that described in Table 3-6 were added to one of the two pairs of tanks. The same medium, minus the phosphate buffer was added to the other pair of tanks.

Twelve 1.8m Douglas fir poles were removed from cold storage and debarked by hand. A small section 50mm long was cut from the ends of each pole and discarded; the remaining bulk of each pole was cross-cut into equal sized pieces, giving 24 short bolts. Four rows of four incisions were made along the length of every bolt to a depth of 2cm. The arrangement of incisions can be seen in Figure 3-5. Bolts cut from the same pole were assumed to be replicate samples and were carefully labelled for identification later. Two replicate pairs of bolts were set aside as non-sprinkled

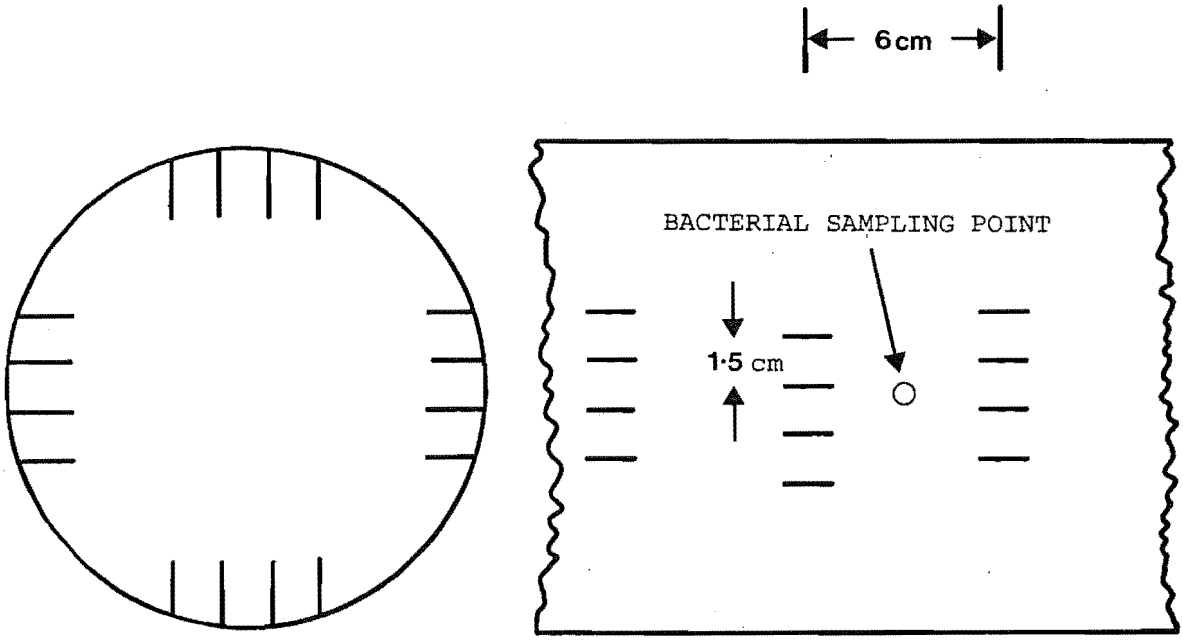


Figure 3-5 Schematic diagram showing the arrangement of incisions made in roundwood poles.

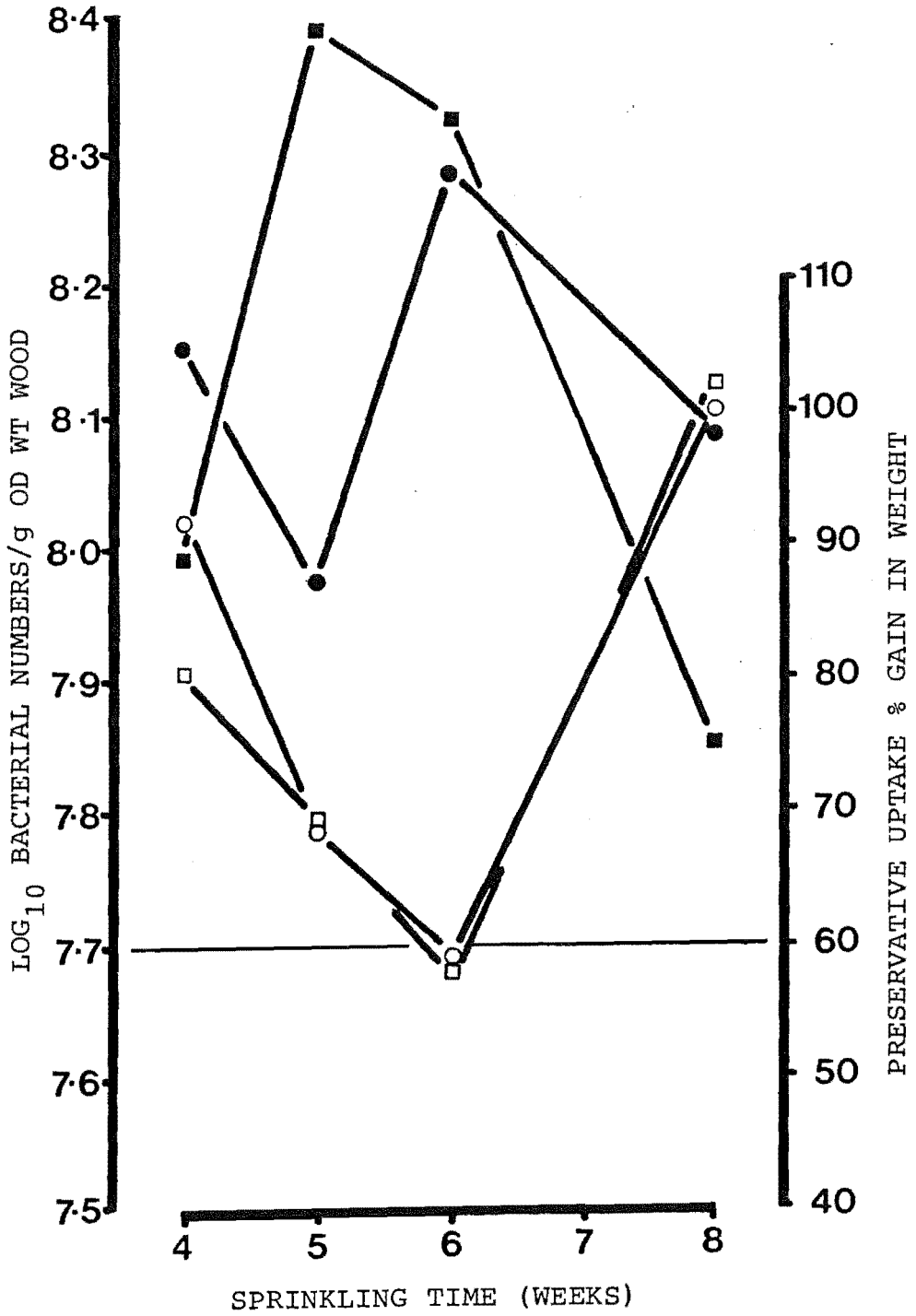
controls. The remaining ten replicate pairs were allocated to the sprinkling tanks so that one replicate was placed in a buffered tank and the other replicate into a non-buffered tank. Each tank was inoculated with 500ml of a three day BPCE culture.

Two replicate pairs of bolts were removed after four, five, six and eight weeks sprinkling. At each sampling time a sterile increment corer was used to remove a small core from the middle of each bolt in between two lines of incisions as indicated in Figure 3-5. Bacteria present in

those cores were counted using the dilution plate technique outlined in Appendix M4. Bacterial counts between replicate bolts and among different sprinkling times were compared on an oven dry weight basis and the results are presented in Figure 3-6. All bolts, including non-sprinkled bolts, were kiln dried under a mild schedule (34°C dry bulb and 29°C wet bulb giving approximately 70-75% r.h.) and conditioned to 20% e.m.c. Once conditioned, bolts were end-sealed with a two part epoxy resin (BOSCRETE 10) and pressure treated with Tanalith NCA using a standard Bethel procedure but omitting the final vacuum (Appendix M6). Preservative uptakes were calculated as a percentage gain in weight for each bolt and the results are presented in Figure 3-6. Bolts were cross-cut into two pieces and the maximum and minimum depths of preservative penetration were measured. Although the minimum depth of preservative penetration is an important determinant of preservative efficacy, it is also useful to have an indication of the proportion of the circumference penetrated to the maximum depth. Such a measurement gives a crude estimation of the eveness of preservative distribution in the timber. Hence the total length of the circumference was measured for each bolt and the areas of maximum penetration were expressed as a percentage of that circumference. Results are presented in Table 3-8.

One of the major contributions of this experiment was that it revealed weaknesses in the methodology. Partial end-seal failure occurred in some bolts and undoubtedly contributed to localised preservative uptake and a greater depth of penetration making those results suspect. One possibility for the failure was that the wood expanded as

Figure 3-6 Bacterial counts and preservative uptake in incised and non-incised Douglas fir at different sprinkling times.



KEY TO SYMBOLS:

- ○ Preservative uptake - buffered
- □ Preservative uptake - non-buffered
- ● Bacterial numbers - buffered
- ■ Bacterial numbers - non-buffered

preservative entered during treatment causing the non-flexible sealant to crack. Sampling the bacterial population with a single increment core gave no indication of the distribution of bacteria from the outside of the stem to the inside. Samples were removed from similar positions relative to the incisions in different bolts. It was assumed that the distribution of bacteria around the circumference and along the stem was even. From a single increment core this assumption could not be verified. Statistical interpretation of the results is definitely impaired by the small number of replicate samples but despite the limitations in the experimental design the results reveal interesting trends.

It is unfortunate that no bacterial sampling of the sprinkled bolts was carried out before the first sampling time at four weeks. The average number of bacteria before sprinkling was as low as 10^5/g oven dry weight of wood. This figure had climbed to 10^8 after four weeks but the behaviour of the bacterial populations before this period is unknown. In the period from 4-5 weeks, bacterial numbers fall in buffered bolts but rise in non-buffered bolts. A peak in numbers occurs for both treatments between 5-6 weeks sprinkling.

The presentation of preservative uptake in Figure 3-6 as a percentage gain in weight is too simplistic and does not take into account differences in log diameter and the width of the sapwood band. The results would have been better expressed as uptake efficiencies (a measure of the preservative uptake as a percentage of that theoretically determined by the void space of the wood). However,

preservative uptake (uptake) did not appear to be increased when buffer was included in the sprinkling medium but uptake was affected by the length of sprinkling time. A basal level of 60% gain in weight was observed in the non-sprinkled control bolts after preservative treatment. After four weeks sprinkling, buffered and non-buffered bolts gained 92% and 81% in weight respectively. Between four weeks and six weeks sprinkling the uptakes in both treatments fell to a level comparable to that of the non-sprinkled controls. Interestingly the low point corresponds approximately to the peak in bacterial numbers. From 6-8 weeks preservative uptake increased again to a 100% gain in weight.

The data presented in Table 3-8 provide a better measure of the preservative treatment performance. Incising clearly improved the depth of preservative penetration. The effect was extremely localised in the non-sprinkled control bolts so that areas of maximum penetration corresponded directly with the incised zones. On average the incising teeth penetrated to a depth of 20-22mm; the preservative did not penetrate deeper. In between the incised zones, the penetration was quite shallow (6mm) giving a 'lobed' penetration pattern in cross section (Figure 3-7). The maximum depth of penetration did not increase significantly after sprinkling except in the non-buffered bolts sprinkled for eight weeks. That result was probably affected by end-seal failure and certainly showed the greatest variation. While the maximum depth of penetration does not increase, the proportion of the circumference penetrated to the maximum depth does, suggesting that tangential, not radial permeability is being improved.

TABLE 3-8 MEAN DEPTH OF PRESERVATIVE PENETRATION IN ROUND, INCISED, SPRINKLED DOUGLAS FIR

SPRINKLING TIME (WEEKS)	TREATMENT	DEPTH OF PENETRATION (mm)		SAPWOOD DEPTH (mm)	% CIRCUMFERENCE PENETRATED TO MAXIMUM DEPTH
		MINIMUM	MAXIMUM		
0	CONTROL	6 (0.1)	21 (1.5)	50 (5)	12 (1)
4	NO BUFFER	10 (0.5)	21 (1)	35 (1)	30 (1)
	BUFFERED	8 (0.5)	20 (1)	35 (1)	22 (2)
5	NO BUFFER	9 (3)	22 (1)	30 (3)	27 (2)
	BUFFERED	10 (0.1)	19 (1)	31 (5)	34 (1)
6	NO BUFFER	12 (0.5)	26 (1)	46 (1.5)	27 (1)
	BUFFERED	14 (0.5)	25 (2)	45 (1.5)	32 (2)
8	NO BUFFER	14 (0.5)	34 (5)	36 (2.5)	40 (1)
	BUFFERED	17 (1)	23 (2)	36 (3.5)	43 (5)

FIGURES IN BRACKETS ARE STANDARD ERRORS OF THE MEAN OF TWO REPLICATES ONLY

In contrast the minimum depths of penetration did increase over the eight week sprinkling period. Visually this resulted in the formation of an even ring of preservative around the stem as the areas in between the incisions 'filled in' (Figure 3-7). The apparent differences between the buffered and non-buffered sprinkling treatments seen in Table 3-8 cannot be substantiated statistically.

It is interesting to speculate at this stage on the mechanism of the increased preservative uptake shown in Figure 3-6 and the corresponding changes in the penetration pattern depicted in Figure 3-7. In the second sprinkling trial using quartered bolts, the preservative uptake after eight weeks was 100% of that theoretically possible in the

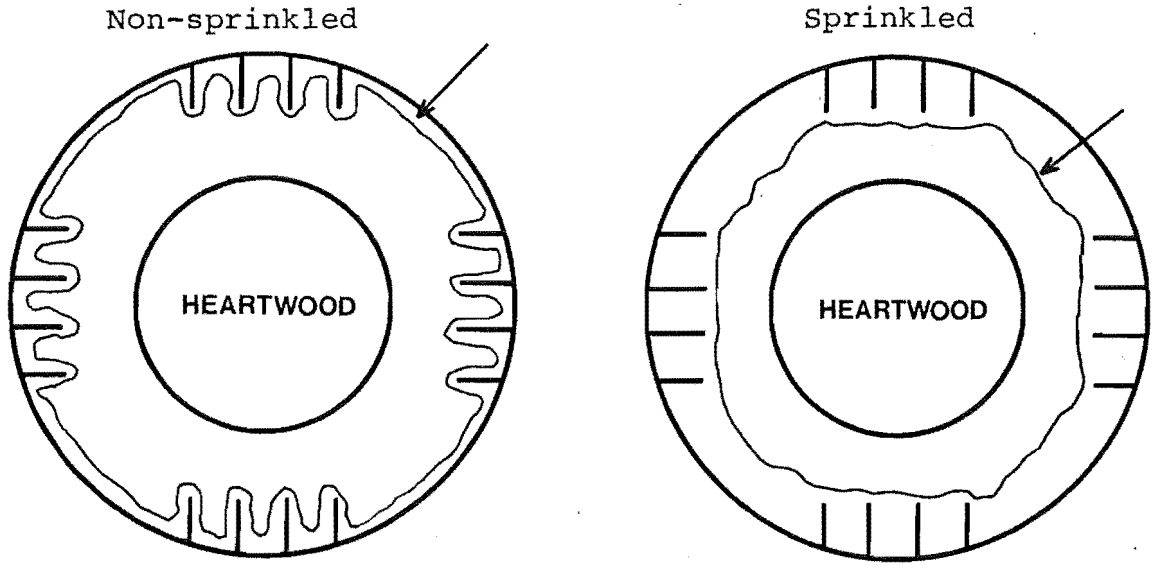


Figure 3-7 Schematic diagram showing the preservative penetration in incised roundwood before and after sprinkling.
Arrows point to preservative front.

sapwood. Visually that appeared as total sapwood penetration. Under identical environmental conditions, with intact round bolts, total sapwood penetration did not occur. One possible explanation for this could be that bacteria can gain access to quartered bolts easier and quicker than they can with round bolts. The exposed tangential faces in quartered bolts can presumably act as deep incisions allowing bacteria into the inner and outer sapwood readily. The normal pathways for bacterial entry into the inner sapwood of round material are more tortuous. The distances involved in axial migration are immense suggesting that transverse migration may be more

important. The evidence presented in Table 3-8 suggests that radial migration is not extensive. Certainly preservative penetration did not increase beyond the depth of incising. Bacteria appear to be able to move most freely in a tangential direction when inside wood. In practical terms this means that deep incising to the heartwood has the potential to assist rapid and uniform bacterial entry to all areas of the sapwood.

CHAPTER 4

METHODOLOGY AND RESULTS FROM SPRINKLING TRIALS USING DOUGLAS FIR

4.1 INTRODUCTION

The results of preliminary sprinkling trials presented in Chapter three and Appendices P1 and P2 established that:

[1] Bacterial colonisation of sprinkled Douglas fir roundwood improves its' permeability. Colonisation of the logs by natural means is slow and tends to be patchy.

[2] The temperature of the sprinkling solution affects bacterial growth. Best results are obtained with temperatures between 20°and 25°C.

[3] The addition of nitrogen and phosphorus to the sprinkling solution promotes bacterial growth. Enhanced bacterial growth leads to greater enzyme activity in the sap which in turn results in more rapid pit membrane degradation.

[4] Bacteria preferentially attack the tracheid bordered pits in the first few weeks of sprinkling. Damage to the tracheid-to-ray pits is not evident until much later.

[5] Shallow mechanical incising assists uniform bacterial ingress at least to the depth of the incision. Further radial penetration beyond the incision is minimal in the course of an eight week sprinkling period. Subsequent preservative penetration is confined to the colonised area and the depth of the incision.

[6] Using quartered roundwood allows bacteria to colonise the entire sapwood band quite rapidly. The exposed radial faces behave like deep incisions on either side of the wood. Total sapwood colonisation by bacteria results in complete sapwood penetration by preservative.

[7] The sprinkling process influences the drying properties of Douglas fir logs. The rate of drying slows in the first few weeks of sprinkling, but then improves after prolonged sprinkling.

Although the preliminary trials demonstrated that bacteria colonising Douglas fir roundwood improve its' permeability, they indicated also that a major limiting factor in the process was the relative inaccessibility of roundwood to bacteria. There is a definite need to examine the mode of bacterial entry into wood in more detail. Similarly the influence of nitrogen and phosphate buffer on permeability improvement requires further investigation. The two nutrients together stimulate bacterial growth, but how they do so is unknown.

Experiments were designed to investigate the relative importance of tangential and radial pathways for bacterial migration into sprinkled roundwood and also to examine the influence of nitrogen and phosphate on the process.

The investigation was divided into two sections; the first section examined the influence of deep incising, allowing the bacteria tangential access to the wood, whereas in the second section bacteria were allowed only radial access to the wood. The effects of nitrogen and phosphate were examined in both cases. It was hoped that a relationship could be established between time and distance moved by the bacteria. Such a function would be useful for predicting the minimum sprinkling time required to achieve adequate preservative uptake.

For the sake of clarity the following section on methodology refers to the incised material only. The methods

used for non-kerfed material are essentially the same as those for incised wood with a few exceptions (fully covered in Section 4.3).

4.2 METHODOLOGY

A basal mineral medium was prepared by dissolving the following salts in distilled water at the concentrations indicated.

0.0005 % $MgSO_4$
0.0005 % NaCl
0.0001 % $CaCl_2$
0.00001 % $FeSO_4$

One 20 l aliquot of that medium was placed into each of four separate sprinkling tanks (Section 3.3, Chapter three). One tank, treatment 1, was supplemented with 17g/l K_2HPO_4 and 4 g/l KH_2PO_4 giving a solution of pH 7.4. A second tank, treatment 2, was supplemented with 5g/l $(NH_4)_2 SO_4$ giving a pH of 6.9, and a third tank, treatment 3, was supplemented with 17g/l K_2HPO_4, 4g/l KH_2PO_4 and 5g/l $(NH_4)_2 SO_4$ giving a final pH of 7.1. No supplement was added to a fourth tank, treatment 4. These four treatments constituted a 2 X 2 factorial design from which it was intended to examine the effects of nitrogen and buffer individually and also the degree of interaction between the nitrogen and buffer treatments.

Eight 1.8 metre poles representing the top logs from eight different trees were removed from cold storage and debarked by hand. Once debarked, the ends (0.01m) were removed and discarded. Four short bolts, each about 0.4m

long, with a 0.05m piece in between were then cut from each pole, giving a total of 32 bolts in all. The short off-cuts were used to estimate the initial moisture content and density of the timber, to see whether or not those physical properties were affected by prolonged cold storage.

A single, radial saw kerf to the pith (simulating a deep incision) was made along the entire longitudinal axis of each bolt. Each bolt was weighed before sealing the exposed axial faces with a thick layer of petroleum jelly followed by aluminium foil. The purpose of the end-seal was to prevent bacteria and sprinkling solution from entering the bolts in an axial direction. Bolts from six of the original poles were allocated to the four tanks so that one bolt from each pole went into each treatment (i.e. each tank held six bolts, one from each pole, a total of 24 bolts). The eight remaining bolts were set aside as controls.

The four tanks were inoculated by pouring 500ml of a three day BPCE culture (Section 3.3, Chapter three) over the bolts stacked in each tank. The bolts were sprinkled continuously for the duration of the experiment. When necessary the fluid level in each tank was topped up with tap water. At weekly intervals for six weeks, one bolt was removed from each tank for analysis. To reduce the inherent variability in the timber, care was taken to select only those bolts which came from the same pole.

Once removed from the tanks, the petroleum jelly and aluminium foil were scraped away and discarded. All bolts were then weighed immediately to determine any change in moisture content from the green state. The following characteristics of each bolt from each sprinkling treatment

were examined:

[1] Moisture content and density
[2] pH
[3] Enzyme activity
[4] Diffusion of nutrients into the wood
[5] Drying rate
[6] Total bacterial population and its' spatial distribution in the wood
[7] Preservative treatability
[8] Microscopic evidence of bacterial attack

The sampling procedure used to examine those parameters is as follows. One disc 2cm wide and another 1cm wide were cut from the center of every bolt removed from the tanks. The thinner disc was set aside to measure bacterial numbers, moisture content and density, the thicker disc for sap pH measurement and enzyme activity. A schematic diagram detailing the breakdown of a 1.8m pole for experimentation and analysis is presented in Figure 4-1.

4.2.1 Moisture content and density determinations

Moisture content determinations and density measurements were made on the wedge shaped samples ('N1'to 'N8', Figure 4-2) left after the samples for bacterial counts were removed. The methods used are outlined in Appendices M1 and M2.

4.2.2 Measurement of wood sap pH

Measurement of sap pH was carried out as described in Appendix M3, but the actual sampling procedure requires further clarification. It was thought that a pH gradient would be produced in buffer sprinkled bolts and that it

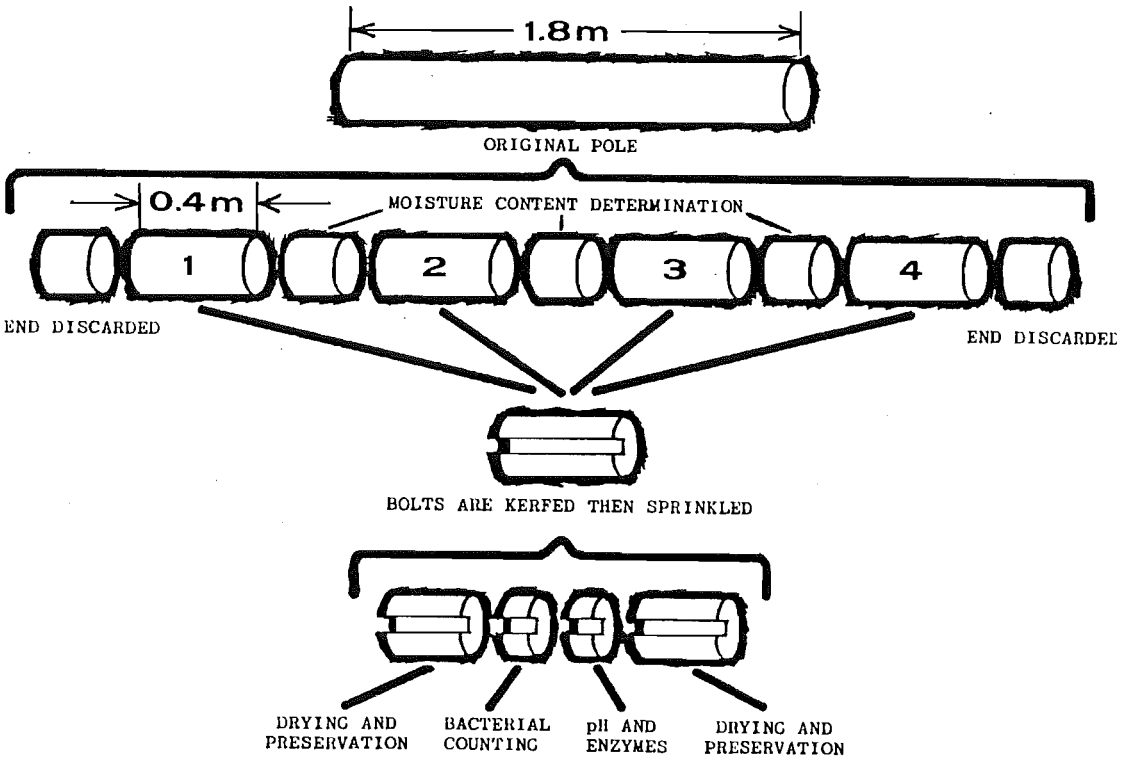


Figure 4-1 Schematic diagram showing breakdown of a 1.8m pole for sprinkling experiments.

would extend tangentially from the kerf inwards. Therefore, the sap pH was measured at increasing tangential distance from the kerf as a function of sprinkling time. Two sampling procedures were employed, one for the buffered treatments (1 and 3) and another for the non-buffered treatments (2 and 4). The procedure for treatments 1 and 3 involved dividing the 1cm discs cut from the sprinkled bolts into nine separate regions, as indicated in Figure 4-3(a). The rationale for selecting the nine regions is as follows: the pH of region (0) was expected to be influenced by the buffer directly and therefore was separated from the inner sapwood; the inner sap, regions (1-4), would

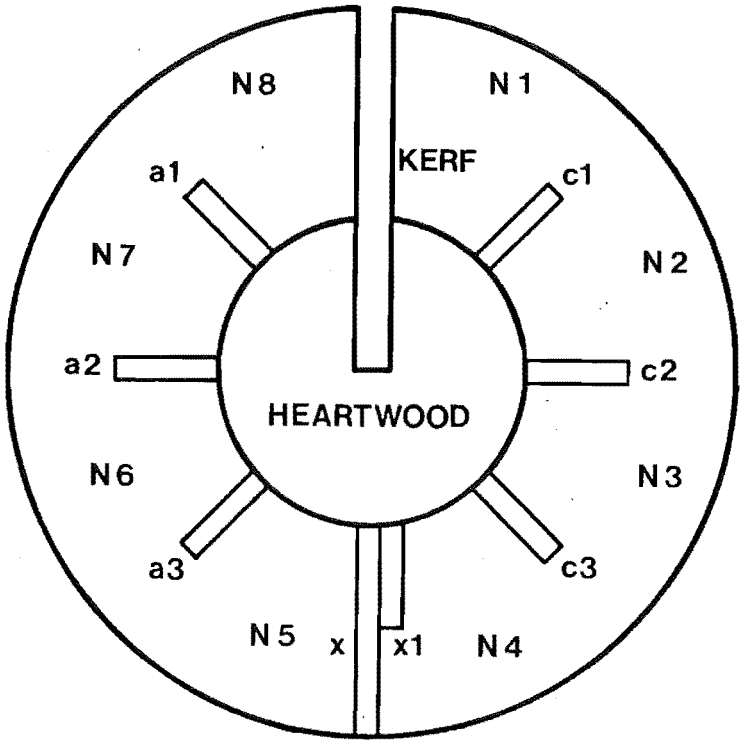


Figure 4-2 Sampling points for assessment of bacterial distribution, moisture content and density in kerfed, sprinkled bolts.

theoretically be affected by only tangential diffusion of the buffered solution. Regions 1(a) and 1(b), 2(a) and 2(b), 3(a) and 3(b) and 4(a) and 4(b) can be considered as replicate pairs of samples taken at increasing tangential distance from the kerf.

The sprinkling solution in treatments 2 and 4 was expected to have a minimal effect on sap pH because the solutions were not buffered. Consequently fewer samples were taken as indicated in Figure 4-3(b). As before, regions 1(a) and 1(b) and 2(a) and 2(b) constitute replicate samples at increasing distance from the kerf.

4.2.3 Enzyme analyses

Enzyme analyses were made on the squeezed sap used earlier for the pH measurements. Thus samples were available from 9 separate zones in treatments 1 and 3 (Figure 4-3(a))

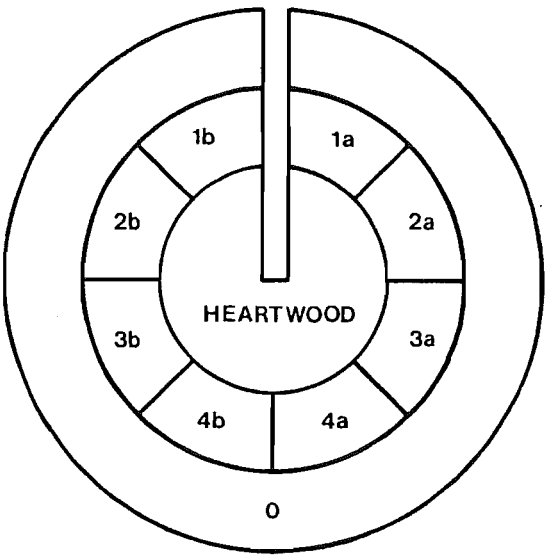


Figure 4-3(a) Sampling regions for sap pH and enzyme determinations in sprinkled bolts - treatments containing buffer.

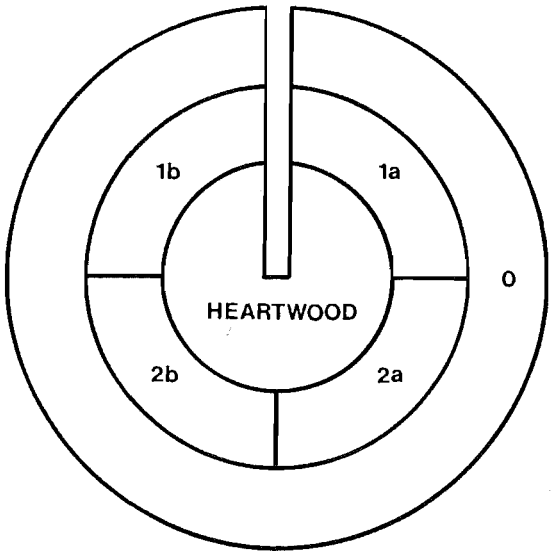


Figure 4-3(b) Sampling regions for sap pH and enzyme determinations in sprinkled bolts - treatments without buffer.

and from 5 zones (Figure 4-3(b)) for those bolts from treatments 2 and 4. It was not possible to perform all of the enzyme analyses from one sampling period in a single session and it was feared that some loss of enzyme activity would occur after prolonged storage. Coloured oxidation products formed rapidly in some samples, so to minimise premature enzyme inactivation the samples were deep frozen until they could be processed. Enzyme analyses were carried

out as described in Appendix M5.

4.2.4 Diffusion of nutrients into sprinkled wood

The diffusion of nutrients into sprinkled wood was assessed using material left over from the analysis of bacterial numbers. Semi-quantitative SEM EDAX X-ray micro-analysis was used to detect levels of phosphorus, potassium and sulphur at various distances from the kerf. Briefly, this involved mounting small wood samples, (originally adjacent to the samples used for bacterial counts) onto aluminium stubs, coating them with carbon to improve conductivity and then bombarding the specimens with an electron beam. The X-rays emitted from the surface of the bombarded specimens were analysed on the basis of energy, wavelength and intensity to determine which elements were present. The technique was unfortunately non-quantitative due to the lack of standards and available computer software. It was however sufficiently sensitive to demonstrate the presence or absence of a specific element.

The distribution of nutrients was found to be quite variable; higher concentrations occurred around the outside edges of the samples and lower concentrations in the centers of the samples. The phenomenon was attributed to relocation of sap during drying. Since only a small proportion of the total area in a specimen could be analysed at one time, five randomly selected areas on each sample were analysed and the mean value was used as an estimate of the amount of element present in the whole sample .

4.2.5 Drying

Details of the methodology and results related to the drying of the sprinkled timber are fully documented in Chapter five.

4.2.6 Bacterial sampling

Preliminary trials indicated that the bacterial population naturally resident in Douglas fir logs was small and uniformly distributed around the entire circumference. It seemed likely that once the bolts were inoculated with non-resident bacteria, a gradient in bacterial numbers would be created. In the initial stages of colonisation it was envisaged that bacterial numbers would be highest in those regions directly exposed to the sprinkling solution, and that the population size would decrease further into the wood. To measure the rate and direction of migration into the wood and to map the spatial distribution of bacteria at different sprinkling times, the cross section of each bolt was divided up into distinct areas.

This was accomplished as follows: Taking the kerf on the thinner disc as a reference point and moving in a clockwise direction around the circumference, three lines were drawn at 45, 90 and 135 degree angles from the kerf. These lines were labelled 'C1','C2'and 'C3' respectively. A similar series of lines were drawn moving in an anticlockwise direction and labelled 'A1','A2' and 'A3'. Small rectangular wood samples, 0.5cm wide, parallel and adjacent to the lines, were removed from the disc with a

surface sterilised axe blade. One side of the kerf was effectively a mirror image of the other, so that two replicate sets of each of the three samples were obtained. Two other samples were taken from the point furthest away from the kerf and labelled 'X' and 'X1' respectively. The sampling areas are indicated diagrammatically in Figure 4-2.

To ensure that any bacteria present in the samples had migrated tangentially from the kerf rather than entering radially, the outer 2.5cm of sapwood was removed and discarded from samples 'A1'-'A3', 'C1'-'C3' and 'X1'. In practice this meant discarding the outer 2-3 growth rings. The heartwood component of each sample was also discarded, so that only the inner sapwood portions remained for analysis of bacterial numbers. Both the inner and outer sap portions of sample 'X' were homogenised together so that bacterial numbers could be compared with those from previous trials.

4.2.7 Preservation

Once kiln dried below fibre saturation point and fully conditioned, the wood was prepared for pressure treatment. The short length of the specimens made it mandatory to end-seal the axial faces. End-sealing was accomplished by applying a coating of 'Boscrete 10', a two-part epoxy resin, and allowing it to cure for at least 24 hours. The timber was weighed and then subjected to a full-cell Bethel treatment without the final vacuum (Appendix M6). Once the treatment was complete, the timber was removed from the

cylinder and reweighed. Preservative uptake was calculated for each bolt from the pre-treatment and post-treatment weights. Uptake results were expressed as a weight gain/cubic metre of timber. The initial moisture content was obtained from the drying study and the moisture content after treatment was calculated from a representative sample cut from the centre of the preservative treated bolts. Moisture content values were used to calculate the uptake efficiencies of the individual bolts using the following equation:

$$U.E. = \frac{M.C.(f) - M.C.(i)}{\text{Max. possible M.C.} - M.C.(i)} \times \frac{100}{1} \qquad (4\text{-}1)$$

where M.C.(i) = initial moisture content
M.C.(f) = moisture content after treatment

The axial faces of treated bolts exposed when removing the moisture content samples were painted with either chrome-azurol or rubeanic acid to determine the extent of copper penetration. A blue reaction with chrome-azurol or a green/black reaction with rubeanic acid indicated the presence of copper. Preservative penetration in a tangential direction was expressed as a percentage of the total circumference occupied by preservative. In practice this involved measuring the angle between the preservative front and the kerf, in both clockwise and anticlockwise directions, adding the two values and then dividing the sum by 360 degrees. Two measurements of radial penetration were made diametrically opposite the kerf with a vernier calliper.

Discs cut from representative bolts were analysed for preservative loading using X-Ray fluorescence (XRF). Small samples were oven dried, finely ground and then pelletized before analysis. The presence of individual CCA components was expressed as a percentage of the oven dry weight. Results were compared with the New Zealand Timber Preservation Authority guidelines to quantify the treatment effectiveness.

4.2.8 Microscopic evidence of bacterial attack

The scanning electron microscope was used extensively to examine sprinkled wood for evidence of bacterial attack. Specimens for examination were removed from areas in the sprinkled bolts adjacent to sites sampled for bacterial numbers. The specimens were either air-dried or freeze-dried prior to examination in a Cambridge Stereoscan 250 microscope. To minimise electron beam damage to fragile pit structures, an accelerating voltage of 20kV was used. Even with this precaution, the more delicate, degraded membranes would disintegrate almost as soon as they were scanned by the electron beam. Despite this limitation, an attempt was made to use the SEM to follow the course of pit degradation as the bacteria moved into the wood.

4.3 METHODS USED FOR NON-KERFED BOLTS.

The composition of the sprinkling tank solutions, bacterial inoculum and allocation of bolts to the tanks were identical to those outlined in Section 4.2 for the kerfed

bolts. As before, two discs were removed from the centre of each bolt and the same parameters were examined. Without the kerf as a frame of reference for measuring tangential distance, most sampling was done in terms of outer and inner sapwood zones as illustrated in Figure 4-4. In practice

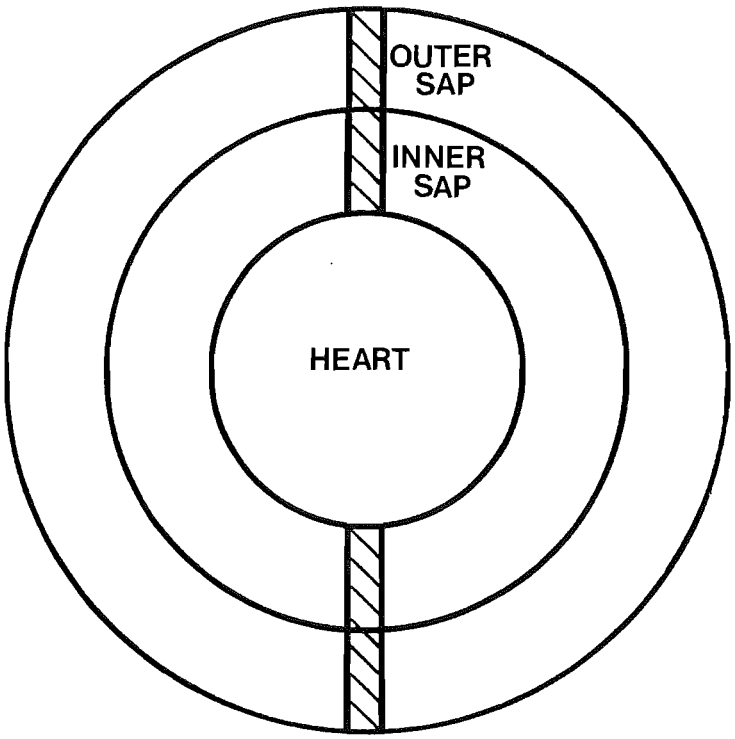


Figure 4-4 Sampling points used in non-kerfed, sprinkled bolts.

outer sapwood was defined as the first 1-1.5cm of wood and inner sapwood the next 1-1.5cm of wood moving in a radial direction from the outside of the stem.

For the purposes of measuring moisture content and density, however, no distinction was made between inner and outer sap. Measurements were made on the complete sapwood zone from samples taken between the shaded areas in Figure 4-4. Enzyme determinations, pH measurements and measurements

of nutrient diffusion were made on inner and outer sap. Samples for bacterial counts were taken from the inner and outer sap at points diametrically opposite each other as indicated in Figure 4-4.

Sprinkled bolts were dried under the same regime as the kerfed material, but the drying rate was not measured. The effects of the sprinkling treatments on the subsequent preservation treatment were assessed through measurement of preservative uptake and radial depth of preservative penetration.

4.4 RESULTS FOR KERFED BOLTS

4.4.1 Moisture content and density measurements in sprinkled wood

In the setting up of these experiments, care was taken to ensure that the timber used was as homogeneous as possible. Variability among individual trees is considerable even when they are selected for their apparent uniformity. For example, in an effort to minimise variability, only the top logs from trees sharing the same microclimate were used in the kerfing experiment (Appendix R1) However, the mean densities of those logs still ranged from 340 to 410 kg/m^3 (Appendix R2). Even within one tree, physical factors such as density and treatability vary from the butt to the crown. The preliminary trials outlined in Chapter three illustrated that extreme variability. In short, the standard, uniform tree does not exist.

Logs sealed in polythene sleeving and stored at 4°C

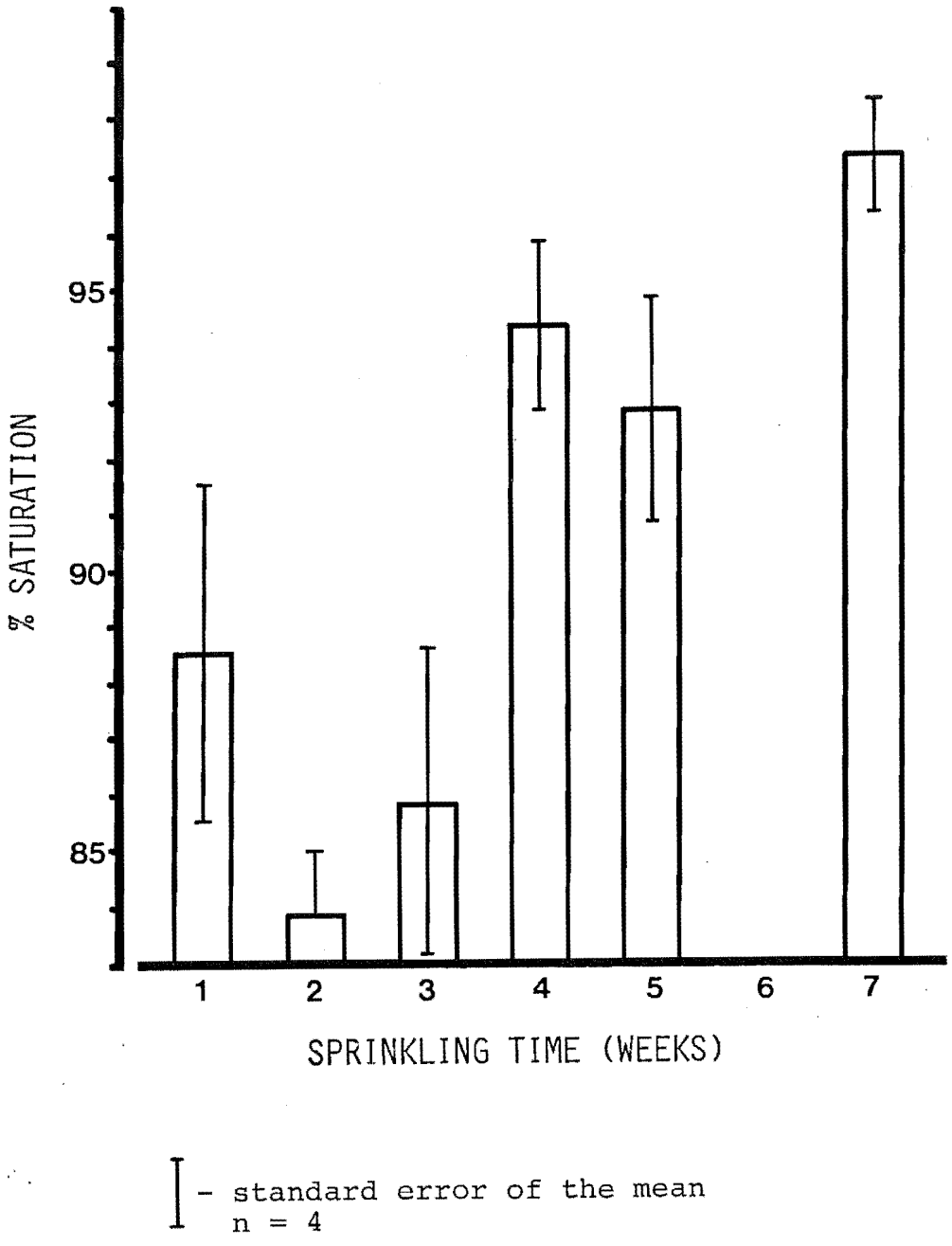
did not dry out. Moisture contents of stored logs were not appreciably different from those of freshly felled logs. There were no significant differences (P=0.01) in the mean sapwood moisture saturation levels among the poles at the start of the experiment.

One of the first measureable effects of sprinkling on the bolts is a gain in weight and a concomitant increase in moisture content. Because that weight gain represents the absorption of sprinkling solution, its magnitude is obviously a function of the moisture content of the bolts just prior to sprinkling and also of the maximum potential moisture content, a value dependent on the available void space in the wood. Therefore, the moisture content data presented in Appendix R3 are expressed as percentage saturation values and not as moisture contents.

Almost all the absorption of sprinkling solution occurred in sap wood. Even though exposed by kerfing, the heartwood showed no significant change in moisture content over the seven week sprinkling period. The small increases in heartwood moisture content indicated in Appendix R3 reflect surface absorption in a narrow zone adjacent to the kerf rather than a uniform increase over the entire heartwood region.

Figure 4-5 shows that absorption of sprinkling solution by sapwood increases with time. There appears to be an initial rise in the first week followed by a drop for the next two weeks. It is doubtful whether or not this is a real phenomenon; certainly the apparent differences in the first three weeks are not statistically significant (P=0.05). The increase in percentage saturation continues

Figure 4-5 The percentage moisture saturation in kerfed bolts at different sprinkling times.



from four weeks onwards until a maximum is reached after seven weeks when the experiment was concluded. Moisture contents in the wood at that stage ranged from 165-190% depending on the density. No significant differences in absorption among the sprinkling treatments could be detected at any sampling time. This would suggest that the absorption of sprinkling solution and the resulting weight gain by the bolts is simply a physical phenomenon associated with filling all of the available void space in the wood.

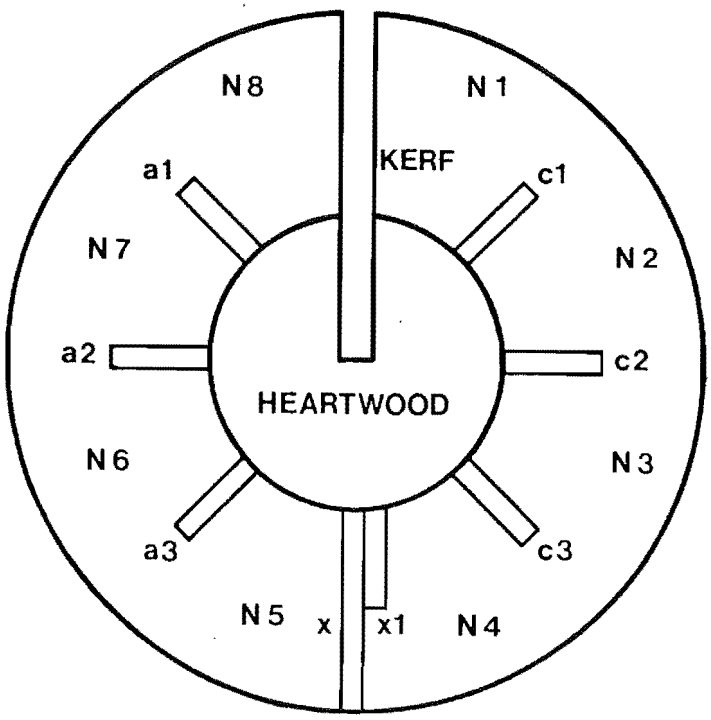
4.4.2 Absorption of nutrient salts

Diffusion of nutrient salts into the timber occurred at the same time as the sprinkling solution was absorbed. SEM-EDAX micro-analysis proved to be an excellent tool to follow the diffusion even though the results were not quantitative. The accumulated data from those analyses are presented in Appendix R5, but a clearer picture of the results can be seen in Figure 4-6. The results for sprinkling treatment 3 only are presented because that treatment alone contained all of the nutrients tested in this experiment. There would be little point in analysing potassium accumulation in sprinkled bolts when the sprinkling solution did not contain potassium (e.g. treatment 4). Figure 4-6(a),(b) and (c) show the migration of potassium, phosphorus and sulphur respectively from the kerf with increasing sprinkling time.

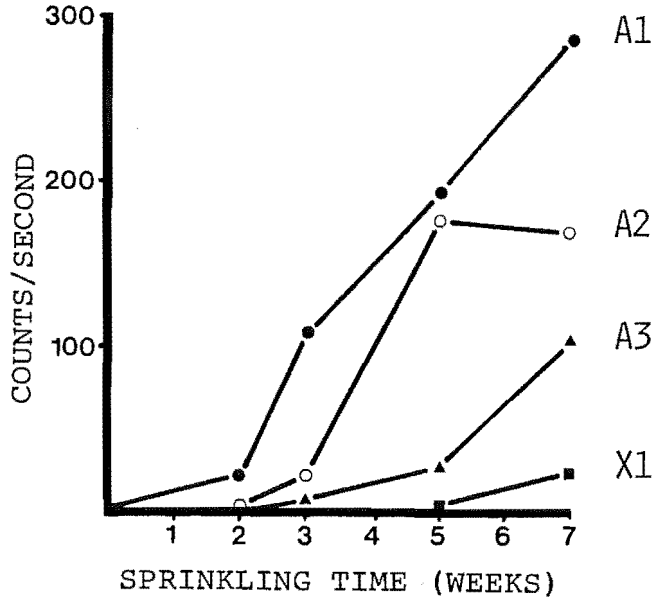
The absolute amounts of each element as measured by counts per second differ (note the differences in scale range between Figure 4-6(a) and (b)). The amounts cannot be

Figure 4-6 Absorption of potassium, phosphorus and sulphur into kerfed bolts with increasing sprinkling time.

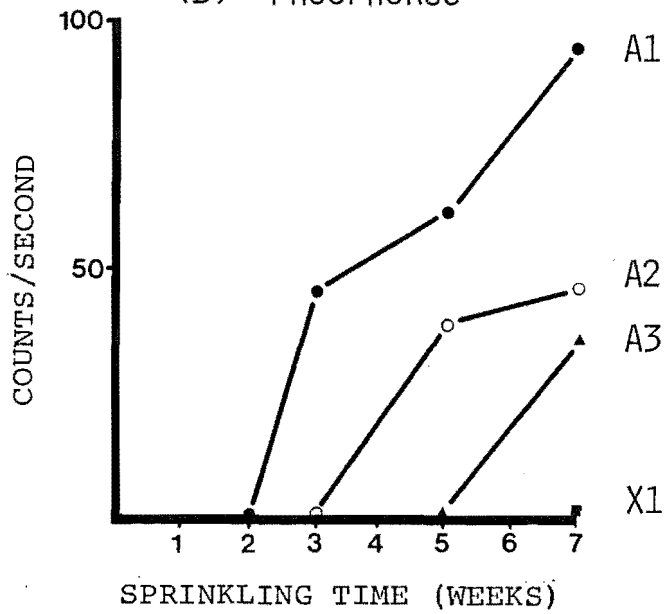
KEY TO POSITION OF SAMPLING POINTS REFERRED TO ON GRAPHS:



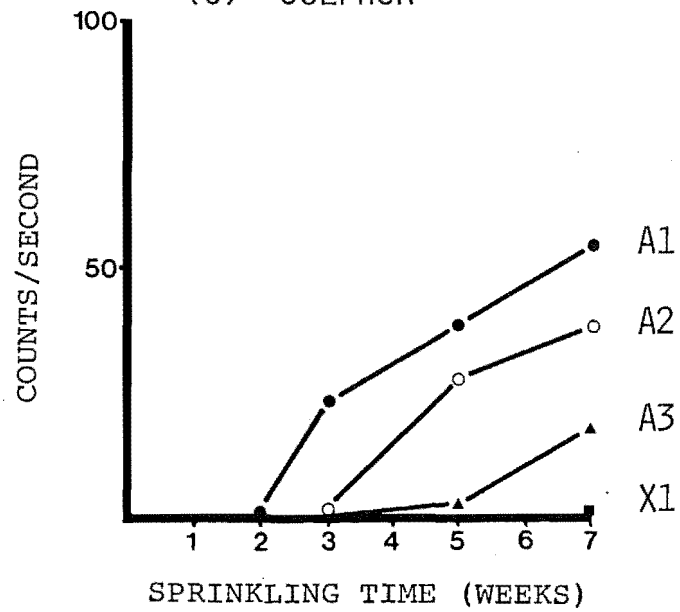
(A) POTASSIUM



(B) PHOSPHORUS



(C) SULPHUR



compared directly because they were influenced primarily by the initial concentrations available for diffusion into the wood (they were certainly not equal) and also by the accelerating voltage of the electron beam (some atoms need a higher energy level for excitation than others). The trends revealed by the graphs are more important than any absolute values.

Before sprinkling, small amounts of the elements were evenly distributed around the circumference of the bolts. After two weeks sprinkling, a small but significant quantity of potassium had diffused through to point 'A1', 45 degrees from the kerf (Figure 4-6(a)). As the sprinkling time increased a migrating 'front' of potassium advanced deeper into the timber. The process continued and eventually, after 5-6 weeks, potassium was detected at position 'X1' (Figure 4-6(a)). Figures 4-6(b) and (c) indicate that similar phenomena occur with phosphorus and sulphur. It is apparent that a considerable period of time elapses before the entire volume of a roundwood bolt becomes impregnated with a nutrient salts solution applied externally through constant sprinkling. The observation is important because for a nutrient treatment to influence bacteria inside wood it must be present in sufficient quantities to stimulate growth. If the availability of a nutrient is restricted then bacterial growth may be sub-optimal.

4.4.3 Changes in wood sap pH during sprinkling

The phosphate added to the sprinkling solutions in

treatments 1 and 3 served as a nutrient source and also raised and maintained the sap pH at a level favouring bacterial growth and optimal pectinase activity. The concentrations of K_2HPO_4 and KH_2PO_4 used constitute a 0.05M pH 7 buffer. The pH of the wood sap was expected to equilibriate with that of the buffer as the phosphates diffused into the wood. Consequently the pH gradients in treatments 1 and 3 should mirror the phosphate gradients. The sap pH in treatments 2 and 4 was expected to be influenced by the metabolic processes occurring.

Table 4-1 shows the pH of the sprinkling solutions inside the tanks and outside the wood after different sprinkling times. The effects of the buffer alone in

TABLE 4-1 pH VALUES OF SPRINKLING SOLUTIONS SAMPLED WHILE SPRINKLING KERFED DOUGLAS FIR FOR DIFFERENT LENGTHS OF TIME

TANK	TREATMENT	SPRINKLING TIME (WEEKS) 0	1	2	3	4	5	7
1	BUFFER	7.4	7.4	7.4	7.5	7.4	7.5	7.5
2	NITROGEN	6.8	6.1	6.2	6.2	6.3	6.0	6.0
3	BUFFER + N	7.1	6.9	6.7	6.6	6.6	6.6	5.9
4	WATER	7.1	7.5	7.8	7.9	7.9	8.3	7.5

treatment 1 are obvious; the pH remained around pH 7.5 for the seven week sprinkling period. Surprisingly the pH in treatment 3, also buffered, fell from pH 7.1 to pH 5.9. Because treatment 3 also contained nitrogen in the form of ammonium sulphate it is possible that the pH drop was due to the release of H+ ions as the bacteria metabolised the ammonium ions. A similar pH drop can be seen in treatment 2

which also contains nitrogen. The pH of treatment 4 on the other hand remained above pH 7. This was unexpected and was attributed to the weak bicarbonate buffering capacity of the local tap water.

Due to technical difficulties no pH data are available for the expressed sap during the first three weeks of sprinkling. However, data were collected after four, five and seven weeks sprinkling (Appendix R6) and are plotted in Figure 4-7. The scale for the 'X' axes used in each graph represents increasing tangential distance from the kerf in equally spaced, but arbitrary units. Point '0' corresponds to the kerf edge and point '4' to the zone furthest from the kerf.

After removal from cold storage, the sap pH in non-sprinkled Douglas fir poles was found to be approximately pH 5 and relatively uniform around the circumference of the logs. For comparisons with sprinkled wood, the horizontal pH gradient observed in non-sprinkled wood is plotted in Figure 4-7(a).

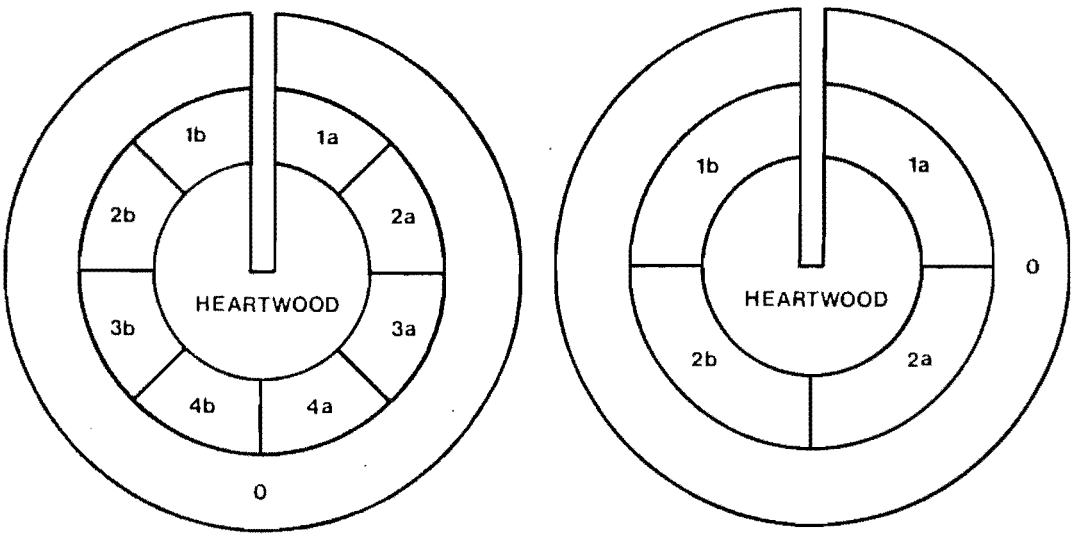
After four weeks sprinkling with a buffer solution (treatments 1 and 3), steep pH gradients were found in the wood. pH values were high next to the kerf and decreased as the distance from the kerf increased. It was suggested earlier the pH distribution in sprinkled wood should correspond closely to the distribution of phosphate (Section 4.4.2). That would appear to be the case. Given sufficient time, the phosphate diffused evenly throughout the wood and the pH also equilibriated resulting in the pH gradient's levelling off. Figure 4-7(b) and 4-7(c) indicate that the pH in treatments 1 and 3, may have been forming such an

Figure 4-7 Changes in the sap pH of sprinkled bolts at different times.

KEY TO SYMBOLS:

- ■ Non-sprinkled
- ● Treatment 1 Buffer only
- ○ Treatment 2 Nitrogen only
- ▲ Treatment 3 Nitrogen and Buffer
- □ Treatment 4 Water only

KEY TO SAMPLING POINTS:



Bolts sprinkled with buffer Bolts without buffer

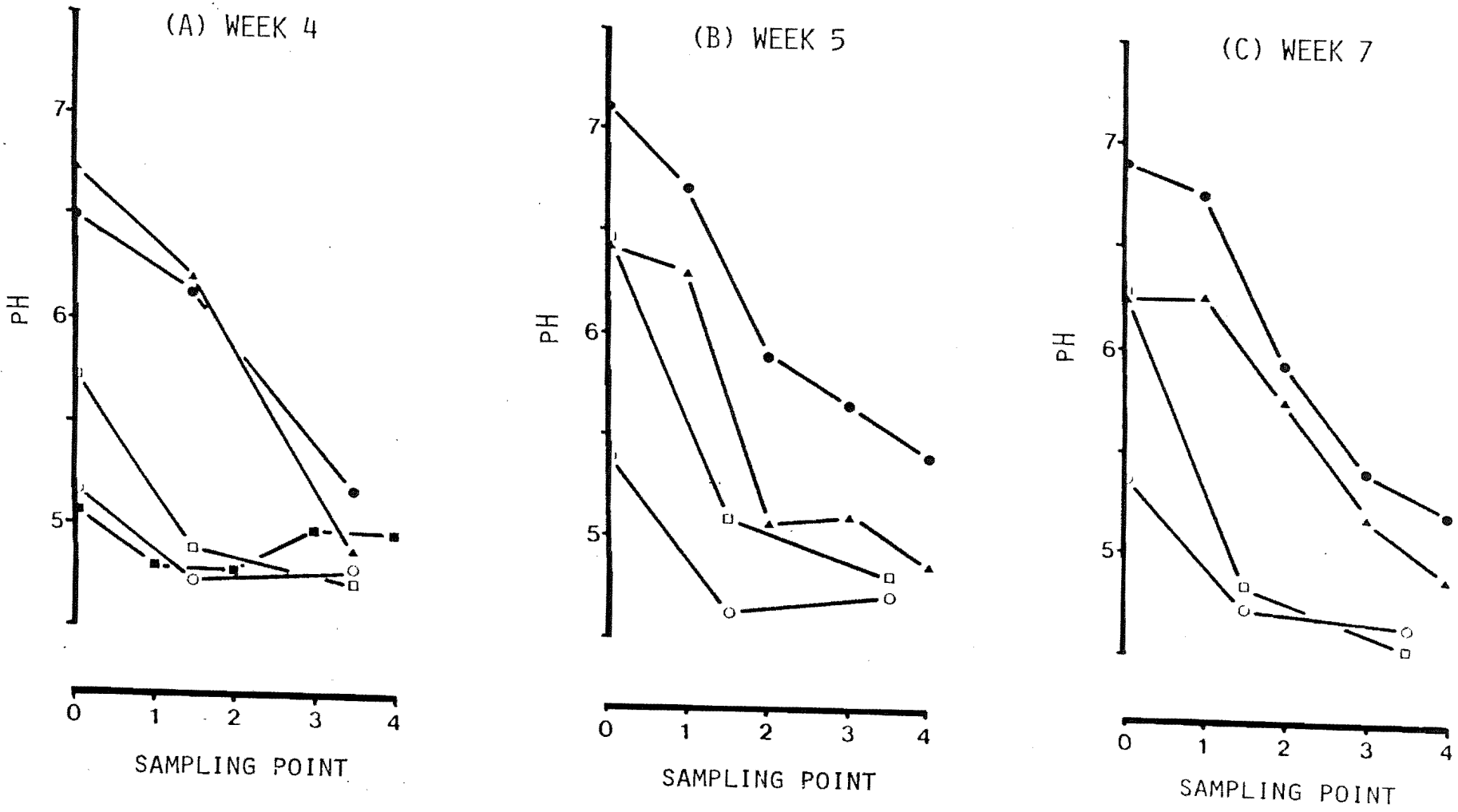


Figure 4-7

equilibrium. Note that the pH from position '0' to position '1' in treatment 3 was approximately the same after seven weeks sprinkling. It is evident that a considerable time period is required before the pH will equilibriate over an entire log under sprinklers.

In the absence of buffer (treatments 2 and 4), the natural pH does not change appreciably after four, five or seven weeks sprinkling (Figure 4-7(a),(b) and (c)). The surface pH in bolts sprinkled with water only (treatment 4) increases from four to five weeks sprinkling but there is no measurable change deeper in the wood. Presumably that increase was due to the weak buffering capacity of the tap water used to top up the sprinkling solution. It could be argued that a similar phenomenon should also arise in treatment 2, but that is obviously not the case. An explanation for the different result may simply be that the buffering capacity of the water was too weak to counteract the acidity of the ammonium sulphate added to treatment 2.

4.4.4 Colonisation and migration of bacteria

Bacteria must first colonise wood before changing its permeability. The total number and distribution of bacteria in wood are important factors affecting the magnitude of any permeability improvement. The total number of bacteria in both sprinkled and non-sprinkled wood was estimated from samples removed from position 'X' (Figure 4-3(a)). Raw data are presented in Appendix R7. No attempt was made to separate the total count into individual bacterial species.

Bacterial numbers in bolts immediately after removal

from cold storage were low, a mean value of 6×10^6 bacteria/g of oven dry wood. That value was taken to represent the natural population level. Some of the bacteria isolated are likely to be contaminants from the sampling procedures. It was assumed that the level of contamination was constant among samples and could therefore be ignored. Bacteria were not counted in freshly felled logs; hence, the effect of cold storage on the natural population of bacteria inside Douglas fir logs is unknown. However, it is likely that numbers increase during prolonged cold storage.

The effects of different sprinkling treatments on bacterial numbers over a number of weeks are shown in Figure 4-8. In the first week of sprinkling the bacterial count increases by a factor of 10 in treatments 2 and 4 and by a factor of 100 in treatments 1 and 3. Examination of cultures isolated after one week sprinkling revealed that the increase was due to colonisation by the bacterial inoculum rather than multiplication of the existing population (Section 4.2). Bacterial numbers in each treatment continue to increase from week 1 to week 4, reaching a peak at week 4. All treatments show a 1000-fold increase in bacterial numbers compared to non-sprinkled wood.

Figure 4-9 illustrates the effects of the different sprinkling treatments and the duration of sprinkling on the distribution of bacteria in wood. The graphs show the logarithm of the mean number of bacteria in a sample plotted against tangential distance from the kerf. Comparisons among the mean number of bacteria were made for each sprinkling time and analysed using analysis of variance (ANOVA). Least significant differences (LSD) are presented

Figure 4-8 Bacterial numbers in the outer sap of kerfed Douglas fir at different sprinkling times.

KEY TO SHADING:


1 week sprinkling


2 weeks sprinkling


3 weeks sprinkling


4 weeks sprinkling


5 weeks sprinkling


7 weeks sprinkling

FIGURE 4-8

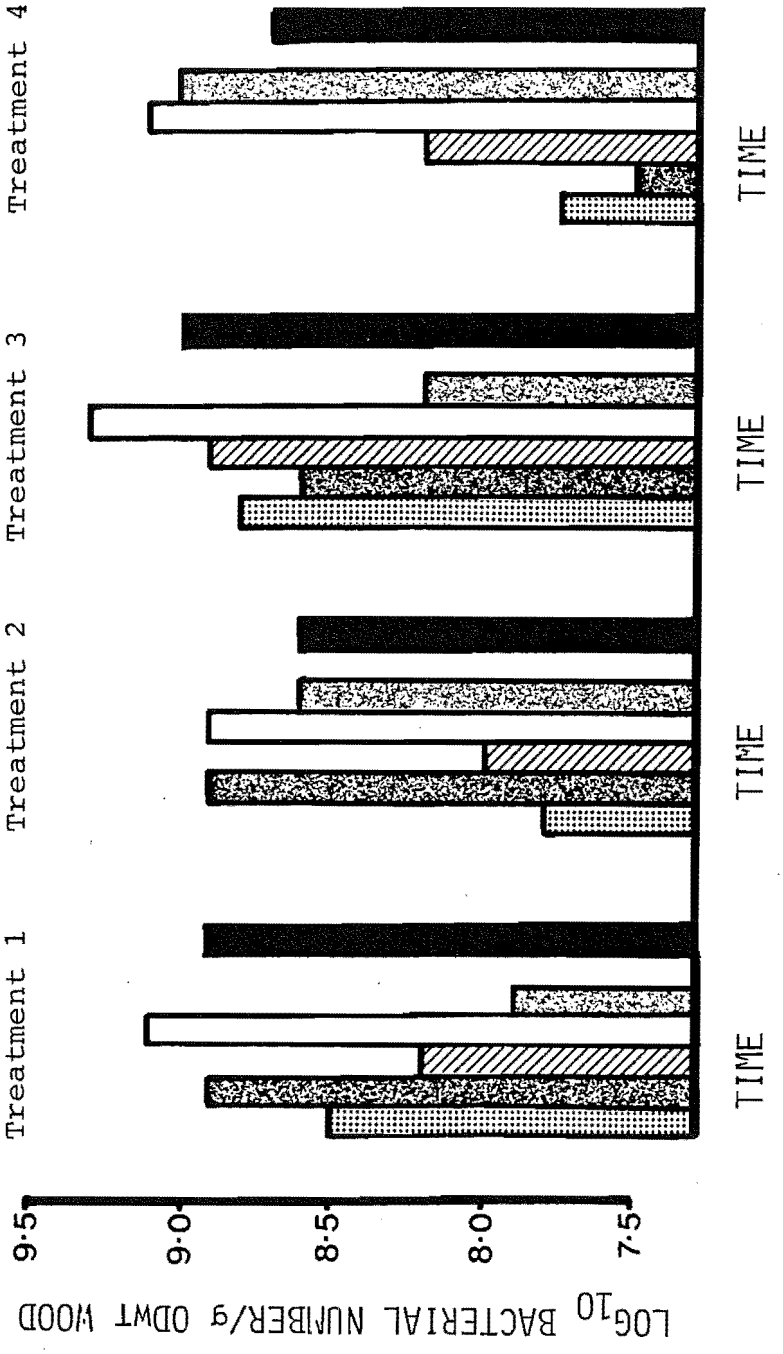
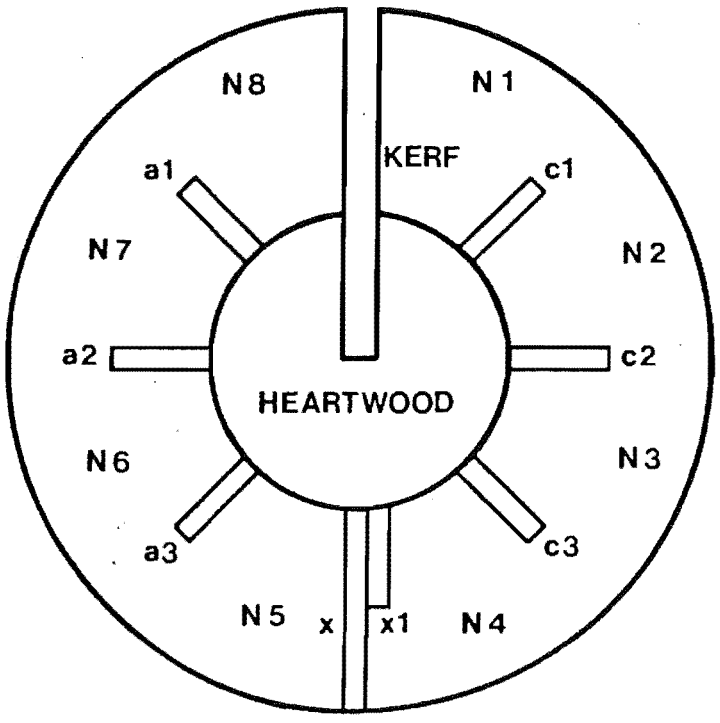

Treatment 1
Treatment 2
Treatment 3
Treatment 4
TIME
TIME
TIME
TIME
9·5
9·0
8·5
8·0
7·5
LOG10 BACTERIAL NUMBER/g ODWT WOOD

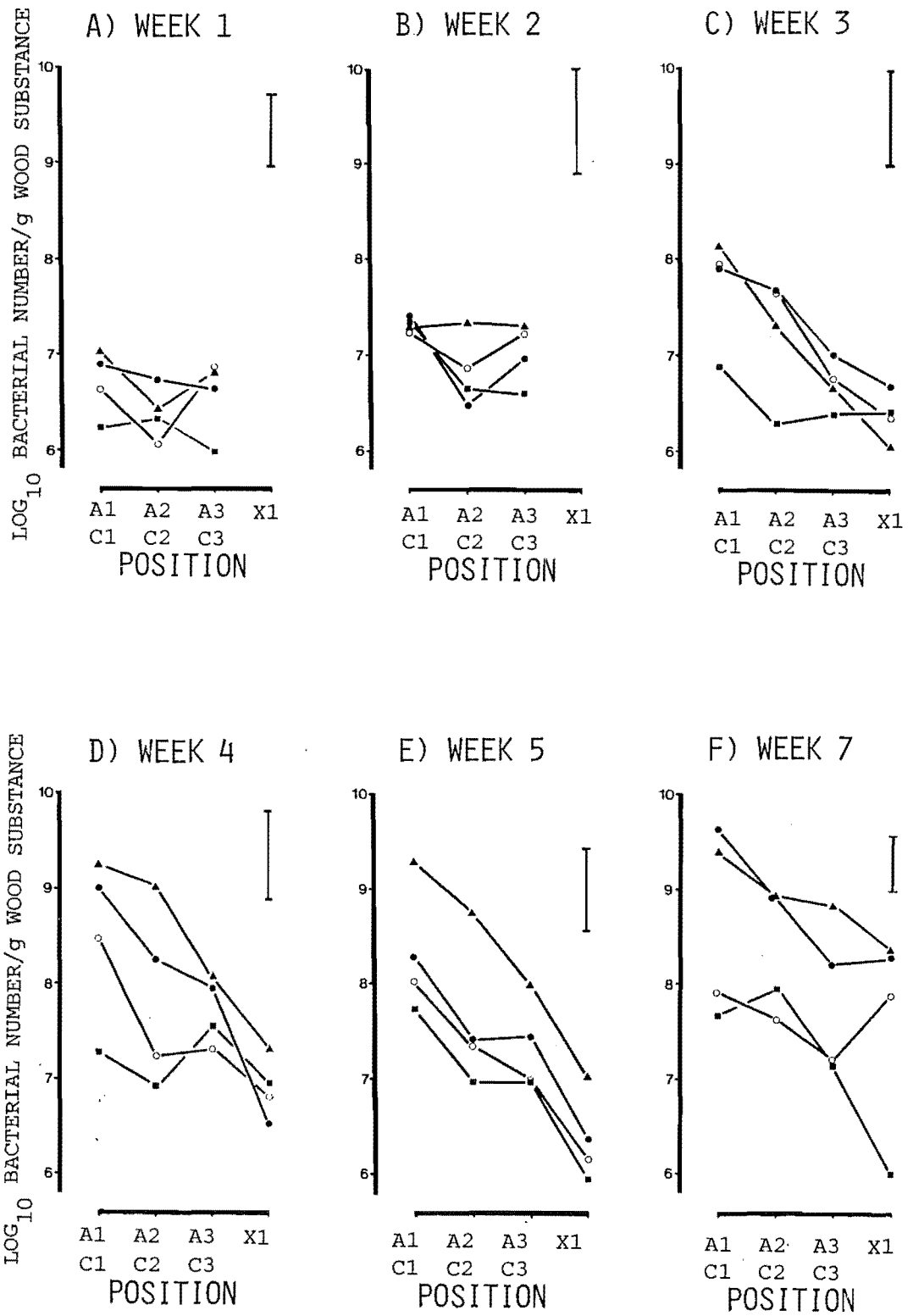
Figure 4-9 Distribution of bacteria at increasing distance from the kerf at different sprinkling times.

KEY TO SYMBOLS:

- ● Treatment 1 Buffer only
- ○ Treatment 2 Nitrogen only
- ▲ Treatment 3 Nitrogen and Buffer
- □ Treatment 4 Water only

KEY TO SAMPLING POINTS:




A) WEEK 1
B) WEEK 2
C) WEEK 3
D) WEEK 4
E) WEEK 5
F) WEEK 7
LOG10 BACTERIAL NUMBER/g WOOD SUBSTANCE
10
9
8
7
6
A1 A2 A3 X1
C1 C2 C3
POSITION

on each graph. Any two means which differ by a value greater than or equal to the LSD can be assumed to be different at a probability of P=0.05.

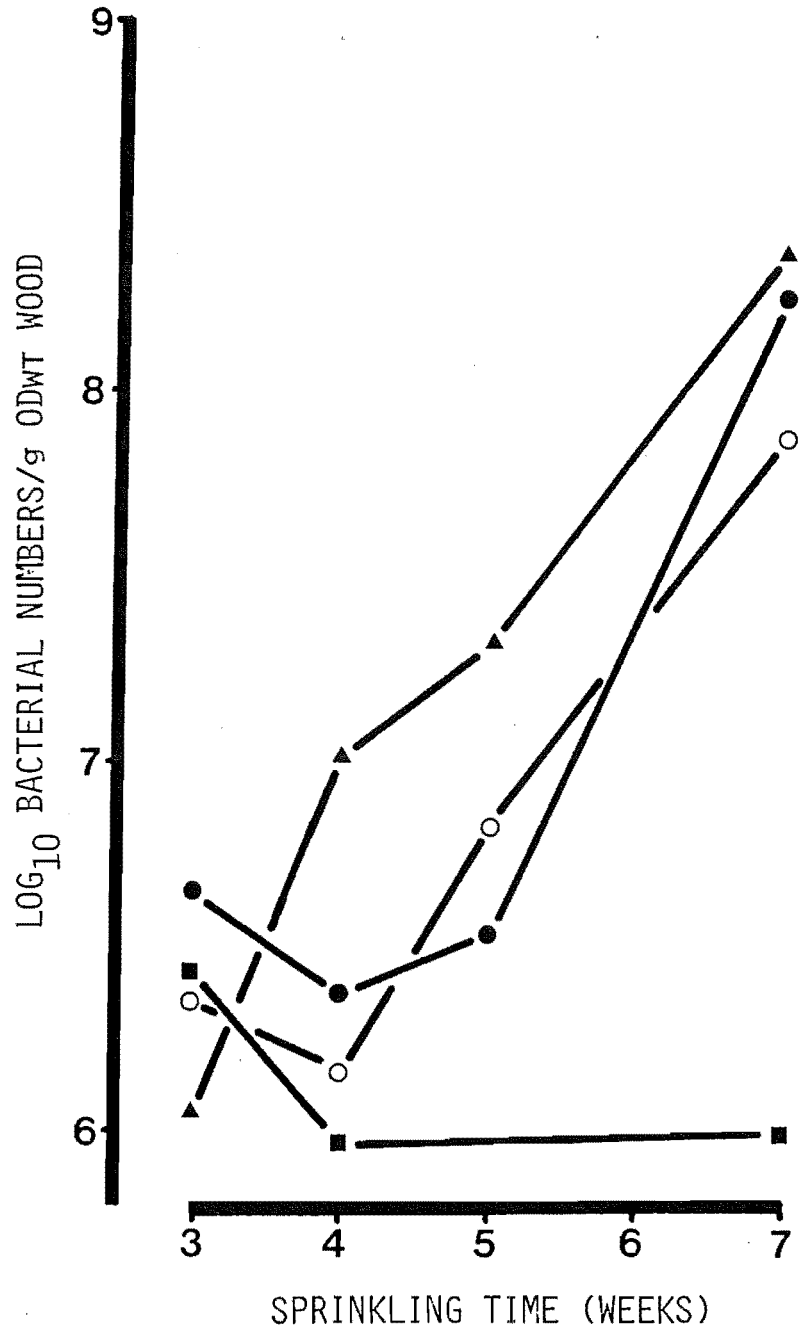
The size of the LSD values indicates that considerable variablity exists in the data; that is because each plotted point is the mean of only two values. Fortunately, the effects of the different sprinkling treatments on bacterial numbers are sufficiently large to make differences self-evident.

Prior to sprinkling, the small bacterial population resident in the timber was uniformly distributed around the entire circumference of each bolt. The natural population level is shown in Figure 4-9(a) as a dotted line. After one week's sprinkling, bacterial colonisation is confined to the surface layers of the timber. Deeper in the wood, there is no measurable increase in bacterial numbers above the intial count in any treatment (Figure 4-9(a)). The first sign of change at position 'A1C1' was noticeable after two weeks' sprinkling (Figure 4-9(b)). No differences among the treatments were recognisable at this stage. By week 3 (Figure 4-9(c)) a bacterial number gradient is evident in treatments 1,2 and 3 (supplemented with nutrients). Bacteria have clearly migrated as far as position 'A2C2'. At week 4 (Figure 4-9(d)), treatment 3 is supporting a greater bacterial population than treatments 2 or 4. Bacteria have migrated to position 'A3C3'. The curve for treatment 1 at week 4 lies above the curves treatments 2 and 4, but it is not significantly different from them at P=0.05. A similar trend exists at week 5 (Figure 4-9(e)). Bacterial numbers are still higher in treatment 3 than in any other treatment,

but the magnitude of the difference is smaller. The experiment was terminated at week 7 and at that time bacterial numbers in treatments 1 and 3 were significantly higher (P=0.05) than those of treatments 2 and 4.

Close examination of Figure 4-9(c), week 3, reveals that there is a linear relationship between the logarithm of the bacterial count and the distance from the kerf for treatments 1, 2 and 3. That same trend can also be observed at weeks 4, 5 and 7. Using the curves for treatment 3 at weeks 3, 4 and 5 as an example, regression analysis demonstrates that there is no difference in their slopes at P=0.05. There are however, differences in their intercepts with the 'Y' axis due to the fact that the total number of bacteria is increasing with time. The homogeneity of the slopes suggests that there was a constant migration of bacteria into the wood during weeks 3, 4 and 5. Perhaps more interesting is the marked drop in slope by the seventh week (significantly different from week 5 at a probability of P=0.05). It is likely that the bacterial population at the surface had reached a maximum, but numbers deep in the wood were still increasing. To demonstrate that increase more clearly, bacterial numbers at position 'X1' opposite the kerf were plotted against sprinkling time as shown in Figure 4-10. There was a linear increase with time from three weeks onwards for treatment 3 and the same trend from four weeks onwards for treatments 1 and 2. No increase can be seen for treatment 4.

Figure 4-10 Bacterial numbers in the inner sap opposite the kerf in D. fir at different sprinkling times.



KEY TO SYMBOLS:

●	Treatment 1	Buffer only
○	Treatment 2	Nitrogen only
▲	Treatment 3	Buffer and Nitrogen
□	Treatment 4	Water only

4.4.5 Pectinase activity inside sprinkled wood

The results of the polygalacturonate trans-eliminase (PGTE) assays made on sap squeezed from wood exposed to different sprinkling treatments are presented below. However, some problems occurred with the assay procedures and they are discussed first.

Squeezed sap stored for longer than a few hours at 4°C or for shorter periods at room temperatures showed a loss of enzyme activity. Loss of enzyme activity coincided with the formation of brown oxidation products in the squeezed sap. The effect was greatest in treatments 1 and 3 which contained phosphate buffer. The problem was not recognised until well into the experiment (after four weeks sprinkling). Storage of the sap for periods of up to one week could not be avoided but, fortunately, the degree of browning and subsequent loss in enzyme activity was minimised by freezing the sap immediately after extraction. The problem deserves mention, because all of the data collected at three weeks sprinkling were so severely affected that it was necessary to ignore them.

Another major problem involved the spectrophotometric assay itself (Appendix M5). Enzyme concentrations in the squeezed sap were obtained indirectly by measuring the enzymatic degradation of a substrate (in this case polygalacturonic acid) to an unsaturated product which absorbs strongly at 235 nm. The assay was very sensitive but that sensitivity proved to be a problem. Absorbance readings fluctuated wildly and it was difficult to obtain a constant rate of reaction. The problem was resolved by

dialysing the sap for at least eight hours before assaying for enzyme activity. The effect of dialysis suggests that some unknown low molecular weight substance present in the sap interferes with the enzyme assay. The identity of the substance was not established, but it could have been calcium. Evidence supporting that role for calcium comes from the fact that calcium is known to form insoluble precipitates with pectinaceous compounds; thus calcium present in the squeezed sap could have precipitated the substrate during the enzyme assay. Precipitates present in the assay cuvette would certainly cause fluctuations in absorbance. Calcium chloride (0.001M) added to the assay mixture was necessary for enzyme activity in dialysed sap, suggesting that calcium ions were removed by dialysis.

PGTE is potentially capable of degrading the pectinaceous components of pit membranes and by so doing improve the permeability of wood. The concentration of PGTE in wood sap and the environmental conditions in sap affecting enzyme activity are two factors affecting the rate of permeability improvement.

The influence of the four sprinkling treatments on the concentration of PGTE in sap squeezed from the outer sapwood of Douglas fir is illustrated in Figure 4-11. Enzyme concentration, expressed as units per millilitre of sap (U/ml), is plotted against sprinkling time for the four treatments. One unit of enzyme is defined as the amount of enzyme capable of liberating luM of product per minute at 25°C. Figure 4-11 shows that a peak in PGTE concentration occurs in all sprinkling treatments after four weeks. The concentration of PGTE after four weeks sprinkling is highest

in treatment 3 (6.7 U/ml of sap) and lowest in treatment 4 (1 U/ml of sap).

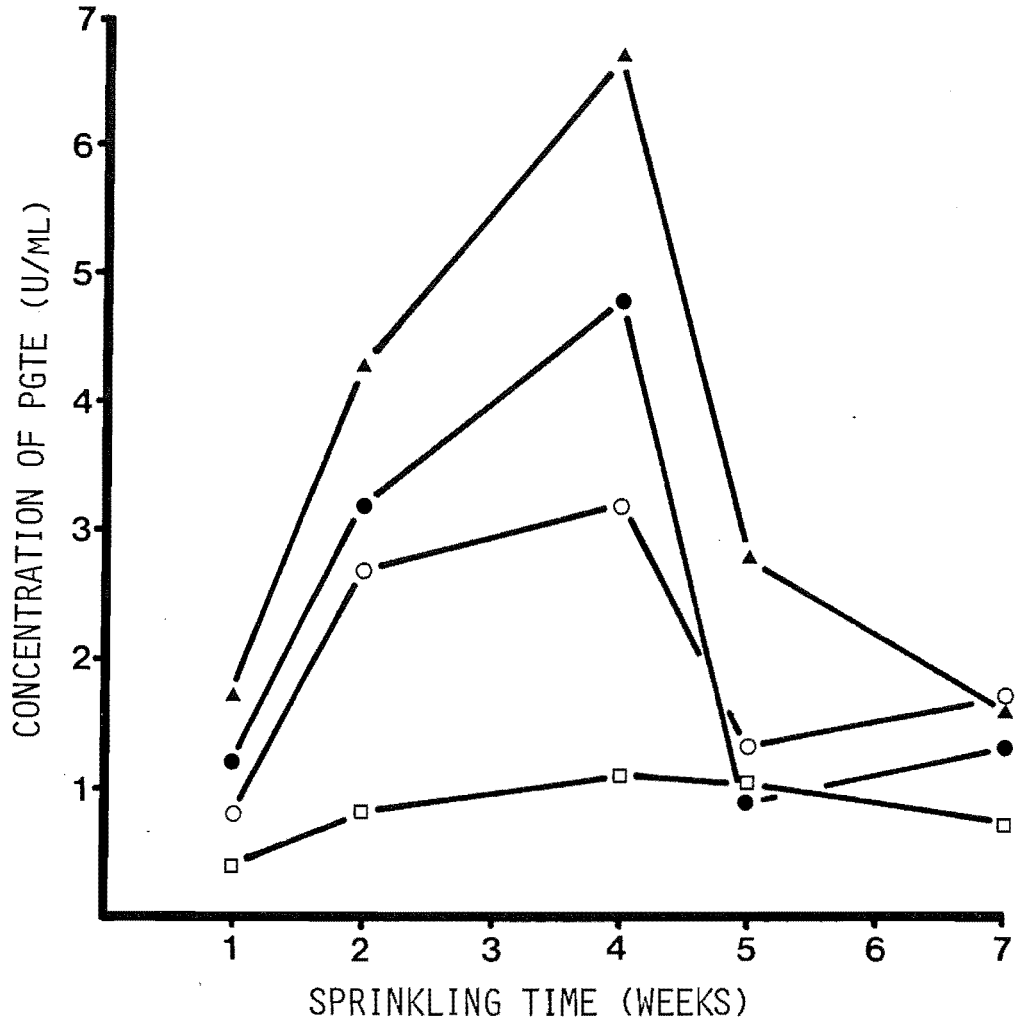
An estimate of the production of PGTE per bacterial cell can be obtained by dividing the enzyme concentration at each sprinkling time by the corresponding bacterial count for each treatment. Such an estimate can be used to establish whether a given treatment influences enzyme concentration by stimulating enzyme production in individual cells or alternatively by promoting bacterial growth. A calculation of the amount of PGTE produced per cell for each treatment was made using the enzyme data in Figure 4-11 and the bacterial population counts in Figure 4-8. The results are presented in Table 4-2.

TABLE 4-2 CONCENTRATION OF PGTE PER UNIT CELL FOR THE FOUR SPRINKLING TREATMENTS AT DIFFERENT SPRINKLING TIMES

TREATMENT	SPRINKLING TIME 1	2	4	5	7
1	0.14	0.35	0.52	0.11	0.14
2	0.11	0.30	0.36	0.15	0.19
3	0.19	0.49	0.72	0.32	0.18
4	0.05	0.10	0.12	0.11	0.08

The trends shown in Table 4-2 correspond closely to those presented in Figure 4-11, which indicates that the production of PGTE paralleled bacterial growth. Indeed, a plot of bacterial number against enzyme concentration for the first four weeks of sprinkling under each treatment (not presented here) indicates an almost linear relationship. It is apparent that the stimulation of PGTE production per cell

Figure 4-11 Concentration of polygalacturonate trans-eliminase (PGTE) in the outer sap of kerfed Douglas fir at different sprinkling times



KEY TO SYMBOLS:

●	Treatment 1	Buffer only
○	Treatment 2	Nitrogen only
▲	Treatment 3	Buffer and Nitrogen
□	Treatment 4	Water only

was greatest in treatment 3 followed in order of magnitude by treatments 1,2 and 4.

In Section 4.2.3 it was stated that discs cut from bolts sprinkled under treatments 1 and 3 were divided into nine zones and those from treatments 2 and 4 into 5 zones for the purpose of assessing differences in enzyme concentration at increasing distance from the kerf. Enzyme concentrations deep inside the wood approached the lower limit of the assay sensitivity and differences between adjacent samples were found to be negligible. Therefore enzyme assays were performed on samples removed from positions '0', '1' and '3' (Figure 4-3), sufficiently far apart to produce measureable differences in enzyme concentration. Enzyme concentrations in the three zones, from 4-7 weeks' sprinkling in each treatment are presented in Table 4-3.

After four weeks' sprinkling, the concentration of PGTE at position '1', in all treatments, was approximately one fiftieth of that in the outer sap at position '0'. No PGTE activity was recorded at position '3'. After five weeks sprinkling, the concentration of PGTE in the outer sap of bolts from treatments 1, 2 and 3 fell, but the concentration at position '1' was approximately double that measured at four weeks. Once again no PGTE activity was measured at position '3'. Enzyme levels in treatment 4 remained constant at positions '0' and '1'. Enzyme concentrations in all treatments continued to increase at position '1' and by seven weeks' sprinkling they had doubled once more. It is also interesting to note that PGTE activity was detected at position '3' again in all treatments, after seven weeks'

TABLE 4-3 CONCENTRATION OF PGTE AT INCREASING DISTANCE FROM THE KERF IN SPRINKLED DOUGLAS FIR

SPRINKLING TIME AND TREATMENT	ENZYME CONCENTRATION (U/ml SAP) AT DIFFERENT DISTANCES FROM THE KERF (REFER FIGURE 4-3) 0	1	3
4 WEEKS T1	4.8	0.09	___
4 WEEKS T2	3.2	0.14	___
4 WEEKS T3	6.7	0.13	___
4 WEEKS T4	1.1	0.02	___
5 WEEKS T1	0.9	0.18	___
5 WEEKS T2	1.3	0.35	___
5 WEEKS T3	2.8	0.25	___
5 WEEKS T4	1.0	0.02	___
7 WEEKS T1	1.3	0.65	0.08
7 WEEKS T2	1.7	0.80	0.11
7 WEEKS T3	1.6	0.50	0.13
7 WEEKS T4	0.7	0.30	0.02

___ INDICATES NO MEASUREABLE QUANTITY OF ENZYME

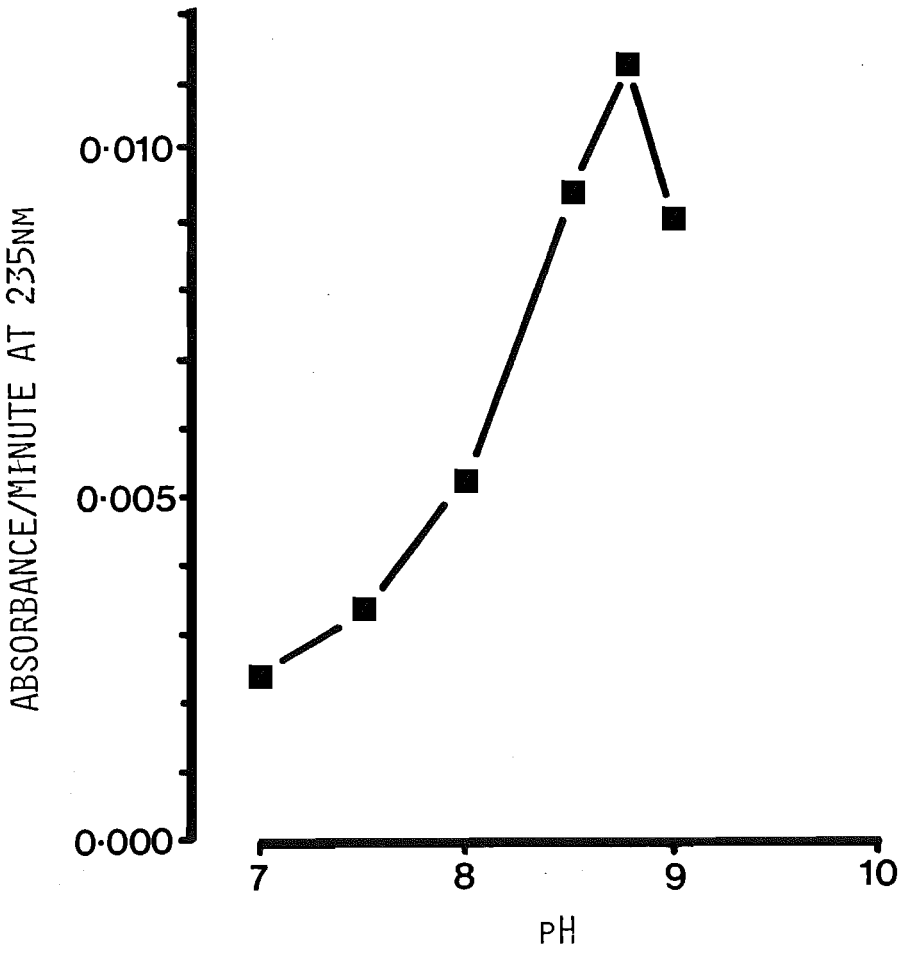
sprinkling.

The significance of the results just presented is difficult to interpret. The absence of any measureable quantity of PGTE at position '3' before seven weeks of sprinkling had elapsed implies that the degradation of pectinaceous material deep inside the wood was negligible. It is apparent that PGTE enzyme molecules did not diffuse freely inside wood ahead of bacterial cells. The data indicate that enzyme production lagged behind bacterial growth. For example, Figure 4-9(d) shows that bacteria had migrated as far as position 'A3C3' (comparable to position '3' in the enzyme analysis) after four weeks' sprinkling, yet no PGTE activity was recorded. By week 5 (Figure 4-9(e)), the bacterial population at 'A3C3' was quite large,

but once again no PGTE activity was measured.

It was mentioned earlier that both the concentration of enzyme and its activity in the sap are important determinants of pectin degradation. Enzyme activity is influenced by pH. The influence of pH on PGTE activity is illustrated in Figure 4-12. A pH optimum is evident at pH

Figure 4-12 Graph showing the pH optimum of PGTE extracted from sprinkled Douglas fir.



8.8. Recalling the sap pH values recorded in sprinkled wood (Section 4.3) it is obvious that under sprinkling conditions

the pH of the wood sap was sub-optimal for PGTE activity. Of the four treatments used in this experiment, treatments 1 and 3 produced the most favourable sap pH for PGTE activity.

4.4.6 Preservative uptake

The amount of preservative uptake depends on the success of the bacterial treatments. Preservative uptake results are presented below.

Detailed raw data showing absolute weight gains by individual bolts after preservative treatment, preservative uptake in kg/m^3 , uptake efficiency and net dry salt retentions are presented in Appendix R8 parts A,B and C. For the sake of clarity only the most important information is provided here.

Preservative uptake in each treatment at different sprinkling times is presented in Figure 4-13. The graph shows quite clearly that sprinkling improved preservative uptake. After only one week's sprinkling, preservative uptake in all treatments was double that of the non-sprinkled controls. As the length of sprinkling time increased, so did the preservative uptake. However, the increase was not linear and the changes were different for the four treatments.

To determine whether the trends observed in Figure 4-13 were significant, statistical analyses were made using analysis of variance. Initially, the mean uptakes for the four treatments at each sprinkling time were compared. Least significant differences plotted at each sprinkling time indicate the significance of any differences among the

Figure 4-13 Uptake of CCA preservative in kerfed Douglas fir at different sprinkling times.

KEY TO SHADING:

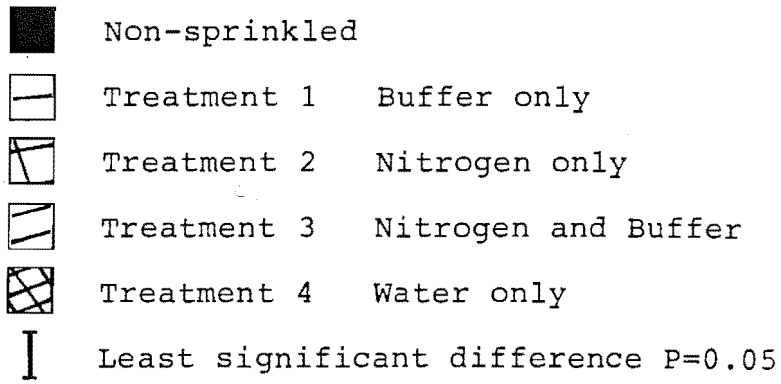


Figure 4-14 Preservative uptake efficiency in kerfed Douglas fir at different sprinkling times.

KEY TO SHADING: as above

Figure 4-13

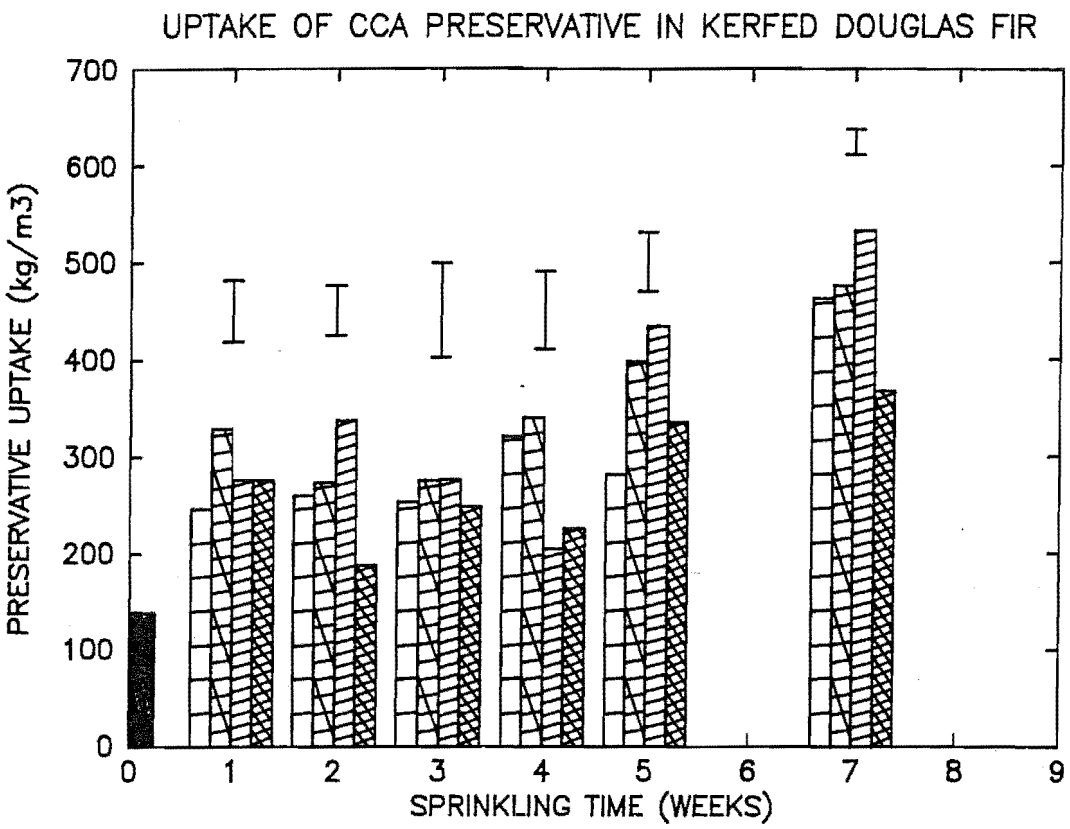
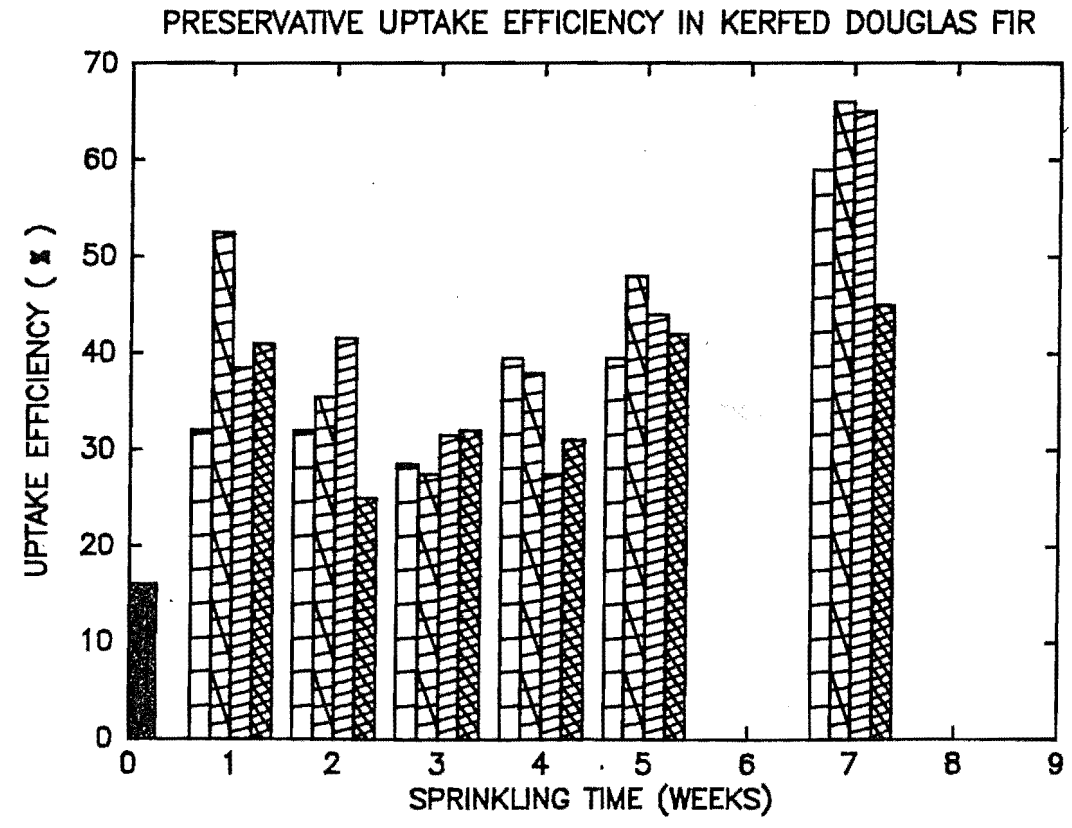


Figure 4-14



treatments.

Essentially, at each sampling time, during the first three weeks, the preservative uptake among the four treatments was similar, although the uptake in treatment 3 at week 2 was higher than that in treatments 1,2, and 4. At week 4, the uptake in treatments 1 and 2 was higher than that in treatments 3 and 4. At week 5, uptake in treatments 2 and 3 was significantly higher than in treatments 1 and 4 and the picture changed once again at week 7. The uptake in treatment 3 was higher than that in treatment 4; and both were higher than in treatments 1 and 2.

It was also important to know whether the changes in uptake within each treatment at different sprinkling times were significant. Measurements of uptake at different times were of necessity made on bolts cut from different logs. So that comparisons were valid, any variation in preservative uptake among bolts sampled from different logs had to be homogeneous. The preservative uptake in non-sprinkled bolts cut from three randomly sampled logs was compared using ANOVA. A summary of the analysis is presented in Table 4-4.

TABLE 4-4 ANOVA SUMMARY OF PRESERVATIVE UPTAKES IN NON-SPRINKLED BOLTS

SOURCE OF VARIATION	SS	DF	MS	F	
AMONG BOLTS	3061.5	2	1530.75	2.084	N.S.
ERROR	6609.5	9	734.38		
TOTAL	9671.0	11	879.18		

The table shows that there is no significant difference in the uptake among different logs which means that comparisons

of the preservative uptakes among different sprinkling times are valid.

The preservative uptakes for each treatment over time show a number of interesting trends. For all treatments there were significant differences in uptake (Figure 4-13). Considering treatment 1 first, the preservative uptakes from weeks 1 to 5 were similar and it was not until week 7 that the uptake became significantly higher than it was at week 1. The uptake for treatment 2 fell from 329 kg/m^3 at week 1 to 275 kg/m^3 at week 3. The drop in uptake was significant at $P=0.05$ and it is not until week 4 that it returned to a level close to that at week 1. The preservative uptake in treatment 3 rose from week 1 to week 2 and then fell significantly from week 2 to week 4. Thereafter it increases considerably and at seven weeks the uptake in treatment 3 was 3.6 times higher than that in non-sprinkled wood. The trend with treatment 4 resembled that occurring in treatment 1. There was no improvement in uptake from one to four weeks' sprinkling, but after five weeks' sprinkling the uptake increased. After seven weeks' sprinkling the uptake in treatment 4 was 2.5 times higher than that in the non-sprinkled controls.

The apparent decreases in uptake observed in treatments 1, 2 and 3 might be disregarded if they were not statistically significant. The reduction in uptake may have been due to high numbers of bacteria blocking the pathways for preservative penetration. To test that hypothesis, an analysis of covariance using bacterial numbers as a covariate was performed on the uptake versus sprinkling time data. However, the treatment means adjusted for bacterial

numbers failed to explain the observed reduction in uptake.

The results of a more refined statistical analysis interpreting the effects of nitrogen, buffer and sprinkling time are presented in Table 4-5. The table shows that adding

TABLE 4-5 PRESERVATIVE UPTAKE IN KERFED DOUGLAS FIR - SUMMARY OF 3 WAY ANOVA WITH NITROGEN, BUFFER AND SPRINKLING TIME AS DEPENDENT VARIABLES

SOURCE OF VARIATION	SS	DF	MS	F	
WITHIN NITROGEN TREATMENTS	39445.3333	1	39445.3333	75.4814	***
WITHIN BUFFER TREATMENTS	1976.3333	1	1976.3333	3.7819	N.S.
WITHIN SPRINKLING TIMES	250762.7500	5	50152.5500	95.9704	***
N X B INTERACTION	3816.3333	1	3816.3333	7.3028	*
N X T INTERACTION	17604.4167	5	3520.8833	6.7375	***
B X T INTERACTION	23023.4167	5	4604.6833	8.8114	***
N X B X T INTERACTION	27830.4167	5	5566.0833	10.6511	***
ERROR	12542.0000	24	522.5833		
TOTAL	377001.0000	47	8021.2978		

nitrogen to the sprinkling solution had a highly significant effect on uptake. It also shows that the effect of buffer was not significant at P=0.05 but that sprinkling time affected the uptake considerably. Unfortunately, nitrogen, buffer and time interact, confounding the analysis. There is a significant interaction of nitrogen with buffer (N X B), which suggests that the influence of nitrogen on uptake was not the same in the presence of buffer as it was if buffer was absent. The N X B means (Table 4-6) indicate that the interaction was interference rather than synergism. For example, the increase in uptake as a result of:

$$\text{buffer without nitrogen} = 303.92 - 273.25$$
$$= 30.67 \text{ kg/m}^3$$

nitrogen without buffer = 348.42 - 273.25
= 75.17 kg/m^3

If the improvement in uptake due to nitrogen and buffer together was additive, the combined uptake should equal:

nitrogen and buffer = 30.67 + 75.17
= 105.84 kg/m^3

In reality the increase was only:

343.42 - 273.25 = 70.17 kg/m^3

or 66 % of the expected value.

TABLE 4-6 N X B MEANS

	B-	B+
N-	273.25	303.92
N+	348.42	343.42

The other significant interactions shown in Table 4-6, N X T and B X T are both fairly predictable from Figure 4-13 and can be interpreted to mean that the effects of buffer and nitrogen were different at different sprinkling times. Similarly a significant three-way interaction of nitrogen buffer and sprinkling time (N X B X T) is also to be expected because of the significant N X B interaction just discussed.

The results of a wood preservation study are often reported as preservative uptake efficiencies: a measure of the amount of uptake as a percentage of the maximum amount that the sample is physically able to absorb. Uptake efficiency standardises uptakes among different logs by allowing for differences in density and hence maximum

possible uptake. Uptake efficiencies calculated from the data in Figure 4-13 are presented in Appendix R8 and graphed in Figure 4-14.

Figure 4-14 shows the same trends indicated earlier in Figure 4-13. It is notable that the uptake efficiencies in Figure 4-14 fell short of 100% even with the excellent uptake levels produced by treatment 3 after seven weeks sprinkling. Uptake efficiency assumes that preservative will be absorbed uniformly over the entire sample. This causes no problems when the samples contain only sapwood or heartwood, but when both are present, especially if the heartwood is non-permeable, the uptake efficiency may be interpreted as being abnormally poor. Usually this difficulty is surmounted by calculating heartwood and sapwood uptake efficiencies separately. When this is done for the uptake data presented in Appendix R8, figures closer to 100% are obtained. For example the following calculations show the uptake efficiencies calculated for treatment 3 after seven weeks' sprinkling, firstly for the whole sample (heartwood and sapwood) and secondly for sapwood only.

Using equation 4-1 (section 4.2.7)

$$U.E. = \frac{\text{M.C. (after)} - \text{M.C. (before)}}{\text{Max. Possible M.C} - \text{M.C. (before)}} \times \frac{100}{1}$$

U.E. (Sapwood and Heartwood)

$$\frac{(137.29 - 12)}{(196 - 12)} \times \frac{100}{1} = 68\%$$

U.E. (Sapwood only)

$$\frac{(163.24 - 12)}{(196 - 12)} \times \frac{100}{1} = 82\%$$

As well as total uptake and uptake efficiency, it is of interest to have a measure of the distribution of preservative within treated wood. In this context, measurements of radial and tangential preservative penetration were made and are presented in Appendix R9. Graphical representation of the same data is shown in Figures 4-15 and 4-16 respectively.

Figure 4-15 shows that after only one week sprinkling, the depth of radial penetration almost trebled from 4.5mm in the non-sprinkled bolts to 12mm or more depending on the sprinkling treatment. Thereafter a gradual decrease in radial penetration occurred until week 4. From week 5 onwards the penetration increased. Variation in penetration was considerable and it is apparent that there were no significant differences in radial penetration among treatments at any sprinkling time. The general trend shown by the plotted points was similar to that of the uptake data in Figure 4-13 except that the preservative uptake at week 7 was much higher than that at week 1, whereas that was not the case for the radial penetration data. Maximum radial penetration was reached in the first two weeks of sprinkling and did not increase further.

Tangential preservative penetration almost doubled in the first week of sprinkling and then fell back to its initial level after two weeks sprinkling (Figure 4-16). From week 3 onwards tangential penetration increased but it was not until week 5 that penetration was higher than that at week 1. Tangential penetration is similar in all treatments until week 7 when the penetration in treatments

Figure 4-15 Radial penetration of CCA preservative into kerfed Douglas fir at different sprinkling times.

KEY TO SHADING:

Non-sprinkled

Treatment 1 Buffer only

Treatment 2 Nitrogen only

Treatment 3 Nitrogen and Buffer

Treatment 4 Water only

Least significant difference P=0.05

Figure 4-16 Tangential penetration of CCA preservative into kerfed Douglas fir at different sprinkling times.

KEY TO SHADING: as above

Figure 4-15

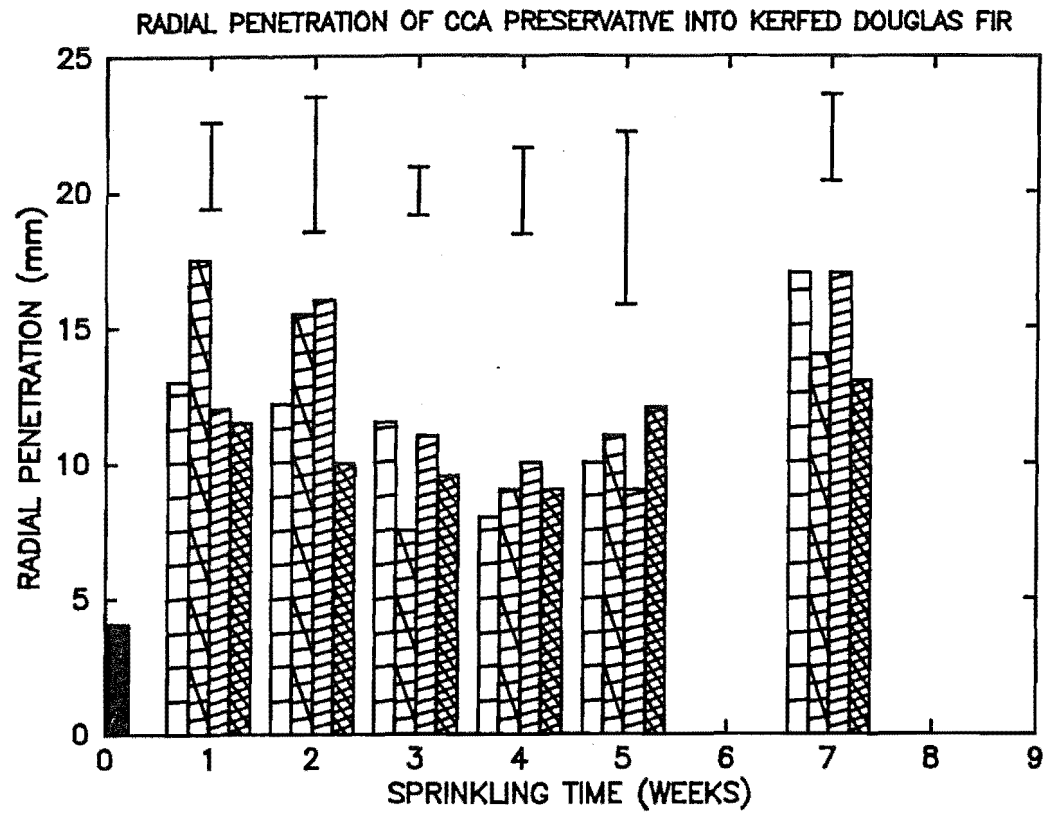
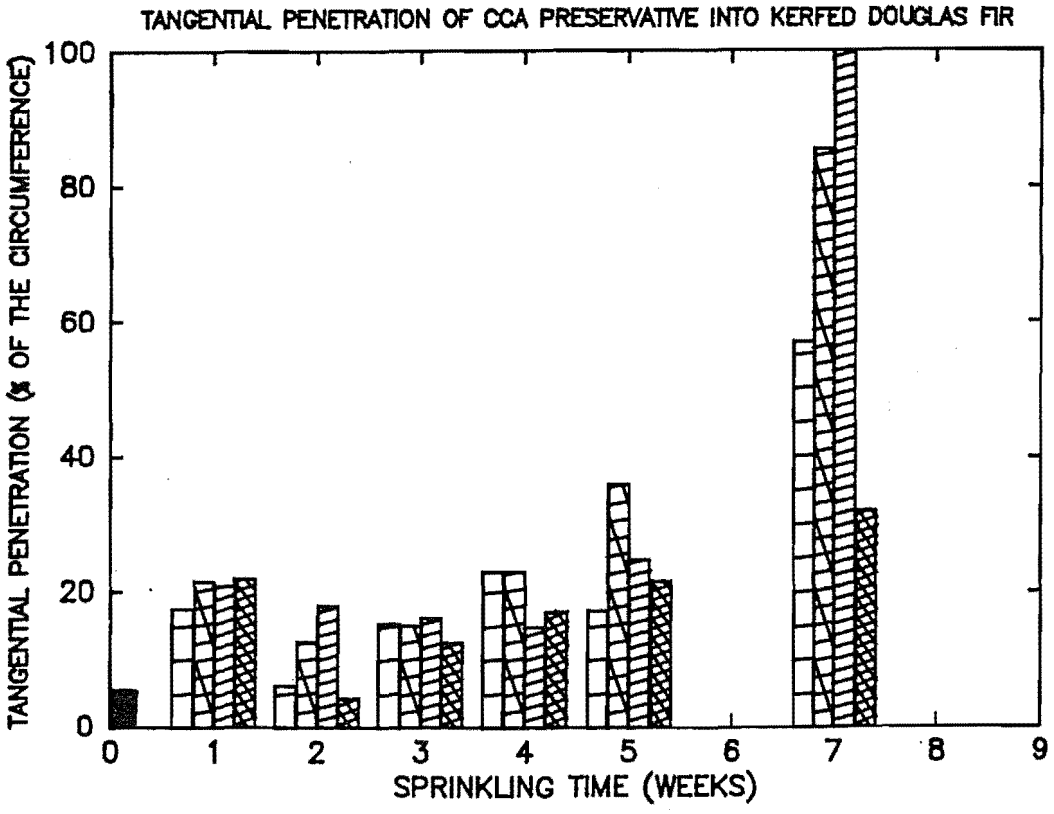


Figure 4-16



1, 2 and 3 were much higher than that in treatment 4.

A regression analysis on radial penetration and preservative uptake, using uptake data as the dependent variate, produced an R-squared value of 0.46. For tangential penetration and uptake the corresponding coefficient is 0.87, suggesting a much better fit for the regression line. This can be interpreted to mean that uptake was more closely related to tangential penetration than to radial penetration.

The penetration of preservative can also be illustrated photographically. Figure 4-17 shows representative cross sections of bolts from each treatment over the seven week sprinkling period. The blue colouration is a positive reaction of chrome-azurol to copper; the orange areas do not contain preservative. At the bottom of Figure 4-17 the remarkably poor preservative penetration in the non-sprinkled bolts is evident. A distinct improvement from this situation was seen after one week's sprinkling. Both radial and tangential penetration had improved. From week 2 to week 4 the poor uptake indicated in Figure 4-16 was evident and it was not until week 5 that there were signs of further penetration. Figure 4-18(a) shows cross sections of bolts after seven weeks' sprinkling compared to the non-sprinkled material. The difference is very obvious, almost total sapwood penetration in treatments 2 and 3 and minimal penetration in non-sprinkled material.

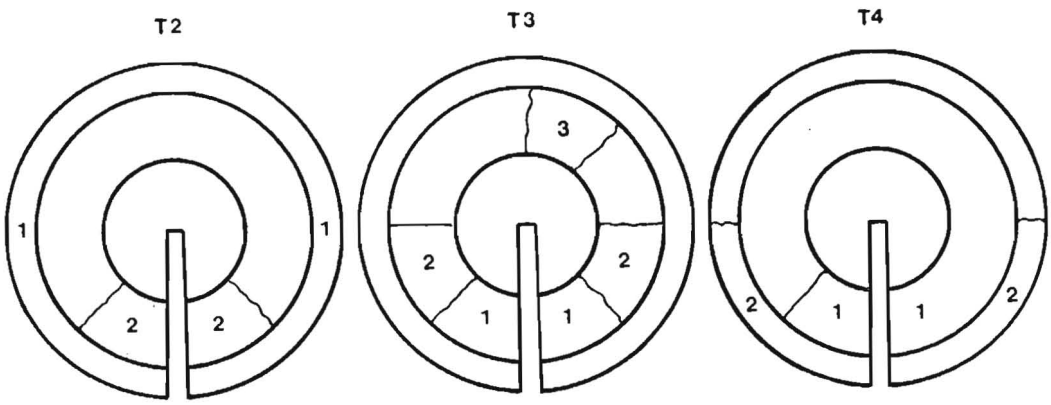
The reason for the colour differences seen in cross sections of the bolts in Figure 4-18(a) was also investigated. The cross-section of the bolt from treatment 2 appears to be solid blue, whereas cross-sections from

Figure 4-17 Photograph showing penetration of CCA preservative in kerfed Douglas fir at different sprinkling times.

Freshly cut faces sprayed with chrome-azurol. Blue colour is positive for copper.

Figure 4-18(a) Photograph comparing CCA uptake in bolts sprinkled for seven weeks with non-sprinkled controls.

Figure 4-18(b) Schematic representation of Figure 4-18(a) showing sampling points for XRF analyses.



treatments 1 and 3 have a mottled appearance; a blue background interspersed with pink. Ordinarily a pink/red colouration with chrome-azurol indicates a copper deficiency.

The distribution of individual preservative components in the treated bolts was measured using X-ray fluorescence and the results are presented in Table 4-7. The sampling points used in Table 4-7 correspond to those in Figure

TABLE 4-7 LOADINGS OF INDIVIDUAL PRESERVATIVE COMPONENTS IN KERFED DOUGLAS FIR SPRINKLED FOR 7 WEEKS

SPRINKLING TREATMENT	POSITION (SEE FIG. 4-18)	ELEMENTAL LOADING (% O.D. WT.) Cu	Cr	As	TOTAL ACTIVE ELEMENTS (%)	POTASSIUM LEVEL (KCPS)
1	N/A	____	____	____	____	____
2	1	0.46	0.52	0.63	1.61	____
	2	0.41	0.41	0.61	1.43	____
	3	0.28	0.28	0.38	0.94	____
3	1	0.23	0.22	0.42	0.87	3.397
	2	0.33	0.38	0.53	1.24	1.563
	3	0.20	0.27	0.27	0.74	4.434
	4	0.29	0.27	0.40	0.96	____
4	1	0.35	0.27	0.50	1.12	____
	2	0.49	0.56	0.64	1.69	____

(____) INDICATES THAT NO ANALYSES WERE CARRIED OUT

4-18(b) and relate to the cross sections of bolts illustrated in Figure 4-18(a).

The chemical balance of the preservative solution before treatment was as follows:

Cu 0.27%
Cr 0.35%
As 0.37%

Total active elements (T.A.E.) 0.99%
Concentration of elements (C.E.) 2.29%

Those percentages are within the New Zealand Timber Preservation Authority (T.P.A.) requirements for copper/chrome/arsenic preservatives. The T.P.A. also specifies the following minimum elemental loadings on an oven dry weight basis to meet a C2 commodity specification for posts and poles in ground contact.

Cu 0.18%
Cr 0.22%
As 0.27%

minimum T.A.E. 0.88%

Table 4-7 shows that only one of the sampled areas, point 3 in treatment 3, is close to failing the required specification. At all other points the T.A.E and elemental loadings are significantly above the prescribed minimum.

Inside treated bolts, the ratio of copper to chrome was higher than that in the original sprinkling solution. The amount of copper in the logs could not be higher than that in the original solution, so the result indicates that there had been a decrease in the amount of chrome.

Table 4-7 also shows that the pink areas (e.g. point 3 in treatment 3, Figure 4-18(a)) correspond to samples

containing the lowest concentrations of copper. The pink areas also contained twice the amount of potassium present in the blue areas. That observation, and the fact that a mottled colour reaction with chrome-azurol occurred only in bolts sprinkled with buffer strongly suggest that potassium phosphate was in some way responsible for localised areas of copper deficiency.

4.5 RESULTS FOR NON-KERFED BOLTS

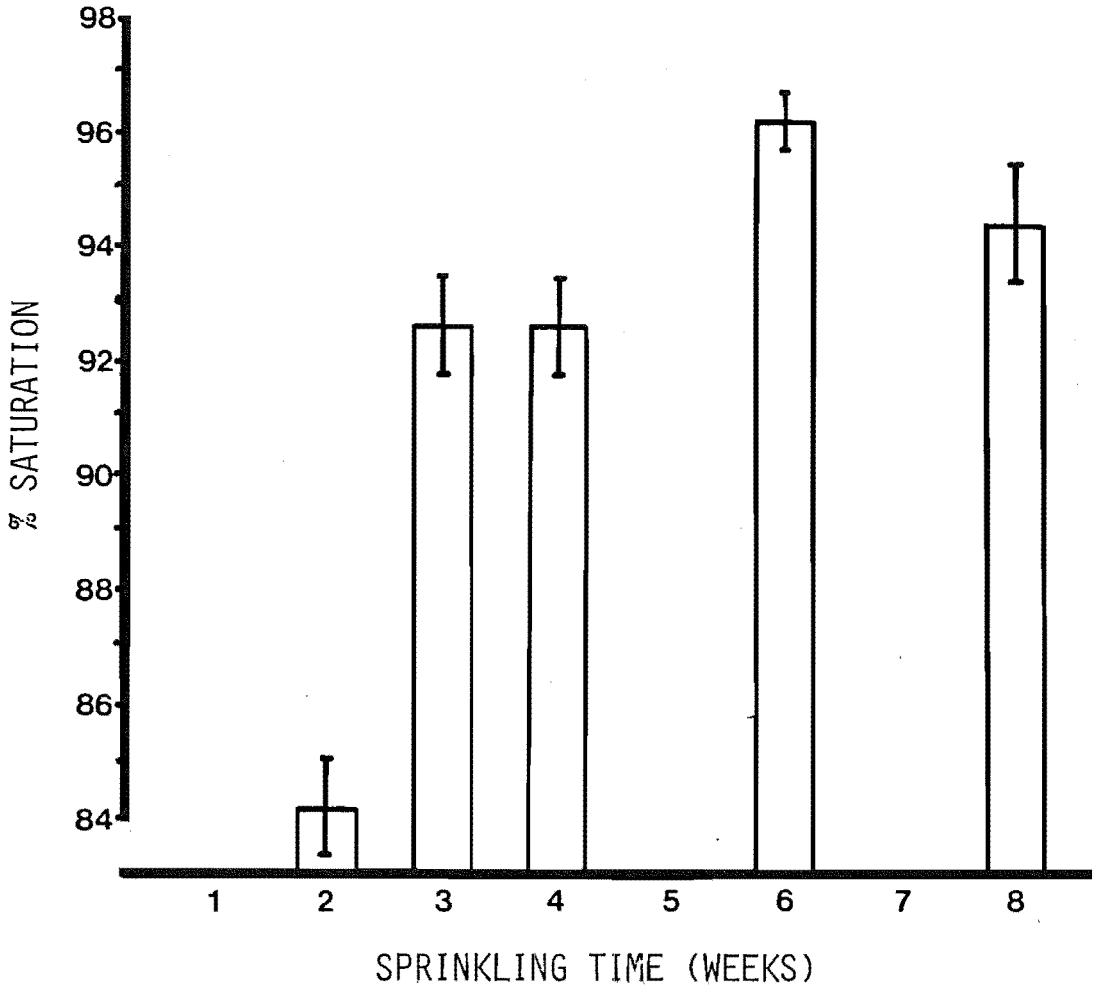
4.5.1 Moisture content measurements

The data presented in Appendix R4 indicate that the weight of non-kerfed bolts increased as a result of sprinkling. Heartwood moisture content did not change in any of the sprinkling treatments. Similar results were obtained with kerfed bolts (Section 4.4.1) and it was shown there that the weight gain was due solely to a rise in sapwood moisture content. Changes in sapwood moisture content are expressed as percentage moisture saturation and plotted against sprinkling time in Figure 4-19. The graph reveals a trend similar to that observed in kerfed logs. The percentage moisture saturation rose rapidly in the first few weeks of sprinkling and then remained at a level close to the theoretical maximum.

4.5.2 Radial migration of nutrient salts into non-kerfed bolts

Nutrient diffusion into non-kerfed wood is illustrated in Figure 4-20. Raw data are presented in Appendix R11. Note

Figure 4-19 The percentage moisture saturation in non-kerfed bolts at different sprinkling times.



I - standard error of the mean
n = 4

Figure 4-20(a) Migration of potassium into non-kerfed Douglas fir.

KEY TO SHADING:

2 weeks sprinkling

3 weeks sprinkling

4 weeks sprinkling

6 weeks sprinkling

8 weeks sprinkling

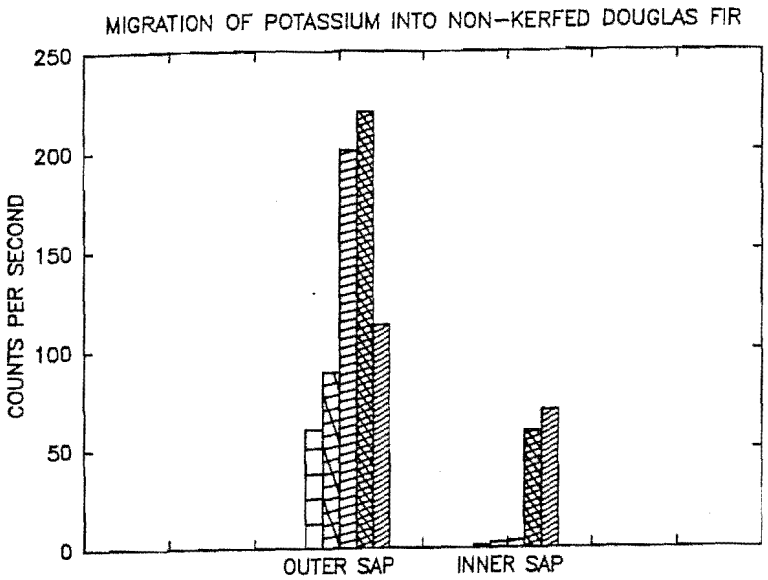
Figure 4-20(b) Migration of phosphorus into non-kerfed Douglas fir.

KEY TO SHADING: as above

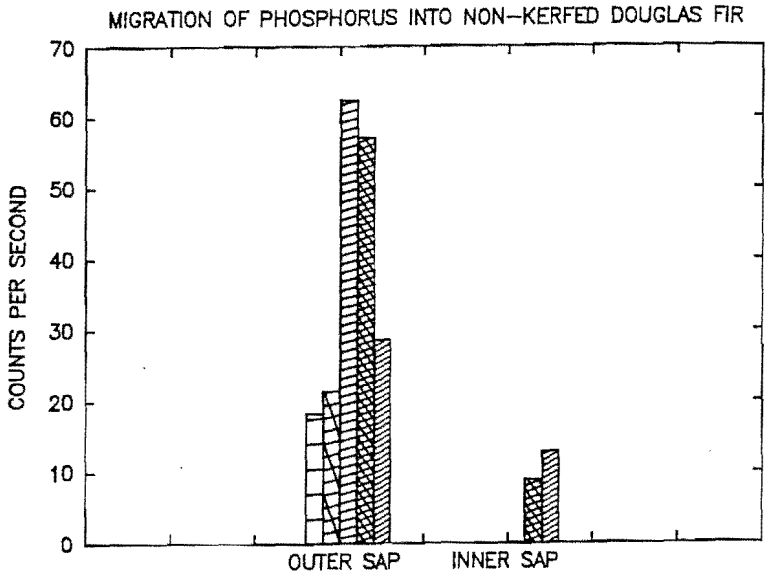
Figure 4-20(c) Migration of sulphur into non-kerfed Douglas fir.

KEY TO SHADING: as above

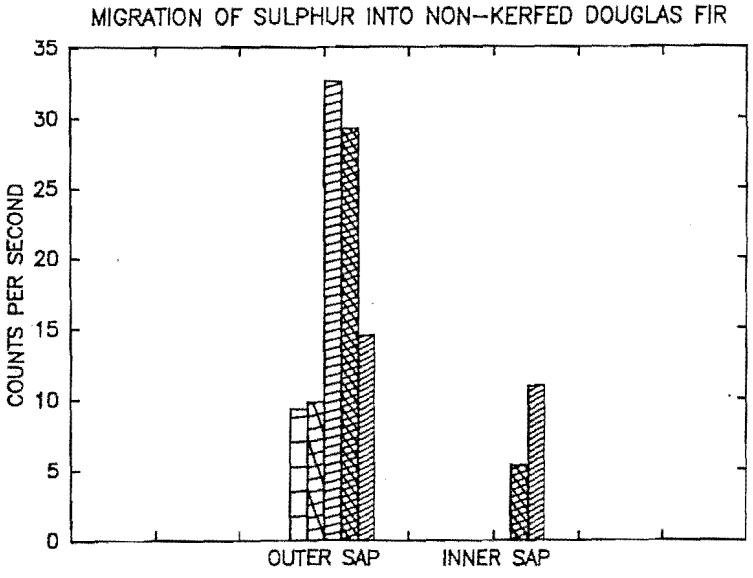
Figure 4-20



A



B



C

that nutrients can diffuse into non-kerfed wood in a radial direction only. In contrast diffusion into kerfed material can occur in both radial and tangential directions.

Figure 4-20 shows that the amount of potassium, phosphorus and sulphur in the outer sapwood slowly increased for the first three weeks of sprinkling. From three weeks to four weeks, a marked jump in the concentration of each element occurred. The concentration of potassium continued to rise until six weeks, but the levels of phosphorus and sulphur fell after four weeks. Figure 4-20 also shows that at least six weeks elapsed before significant amounts of the three elements diffused radially into the inner sapwood zone.

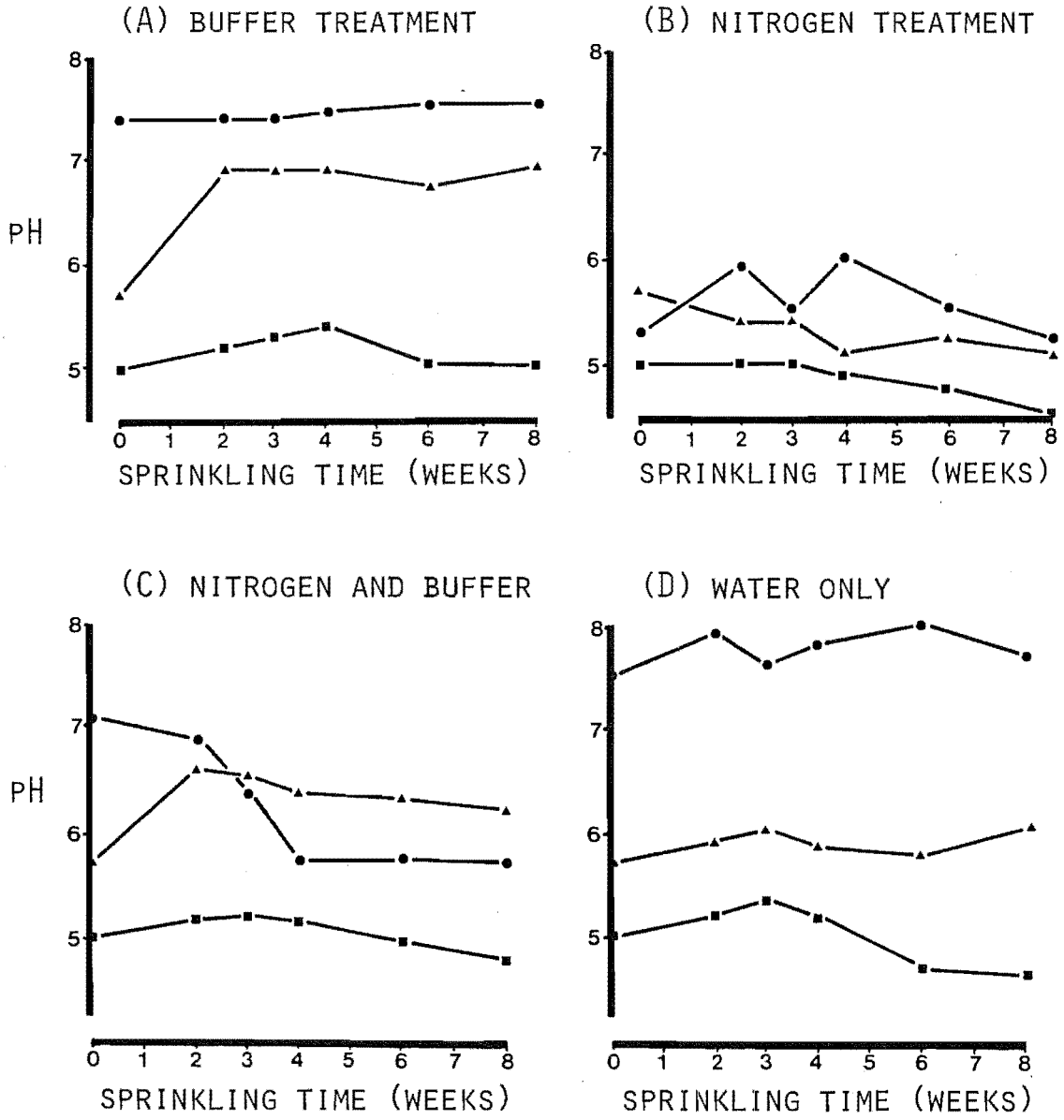
4.5.3 pH changes during sprinkling

Data showing pH changes occurring in the sprinkling solutions and also in sap squeezed from the inner and outer sapwood are provided in Appendix R12 and Figure 4-21.

The influence of phosphate buffer on the pH of the sprinkling solution is obvious; the pH of the solution in treatment 1 (Figure 4-21(a)) remained above pH 7 for the duration of the experiment. The pH of the solution in treatment 2 (Figure 4-21(b)), without buffer, remained below pH 6. Interestingly, the pH in treatment 3 (Figure 4-21(c)) fell from pH 7 to below pH 6 even in the presence of buffer. For reasons already discussed in Section 4.4.3 the pH of the sprinkling solution in treatment 4 remained above pH 7.

The pH of the sap expressed from the inner and outer sapwood zones was closely related to the diffusion of

Figure 4-21 pH of the sprinkling solution and the outer and inner sap expressed from non-kerfed Douglas fir bolts at different sprinkling times.



KEY TO SYMBOLS:

- ● sprinkling solution
- ▲ outer sap
- ■ inner sap

potassium phosphate. There was no substantial change in the inner sap pH of any treatment for the duration of the experiment and, as previously discussed little diffusion of potassium phosphate into the inner sap occurred. In contrast the outer sap pH in treatments 1 and 3 rose from pH 5.7 to pH 6.9 and pH 6.7 respectively, in response to the diffusion of buffer into the wood. The increase was greatest in treatment 1. The outer sap pH in treatment 4 remained constant just below pH 6 while that of treatment 2 fell slightly from pH 5.7 to pH 5.1.

4.5.4 Bacterial growth in non-kerfed wood during sprinkling

Bacterial counts in non-kerfed wood are presented in Appendix R10. Numbers of bacteria present in the inner and outer sapwood in each treatment, at different sprinkling times, are graphed in Figure 4-22.

During the first four weeks of sprinkling, bacterial colonisation of the non-kerfed bolts was confined to the outer sapwood zone. Bacterial numbers in the inner sapwood remained static during that period. Figure 4-22 shows that bacterial numbers in the outer sap of all treatments steadily increased, reaching a peak at week 6. The result compares favourably with the increase in bacterial numbers observed in kerfed bolts (Figure 4-8). Individual treatments did not seem to affect the size of the bacterial population. However, the similarity among treatments cannot be verified statistically because of insufficient numbers of replicates. It is interesting to note that the maximum

Figure 4-22 Bacterial numbers in non-kerfed Douglas fir sprinkled for different times.

(A) 2 weeks sprinkling

(B) 3 weeks sprinkling

(C) 4 weeks sprinkling

(D) 6 weeks sprinkling

(E) 8 weeks sprinkling

KEY TO SHADING:

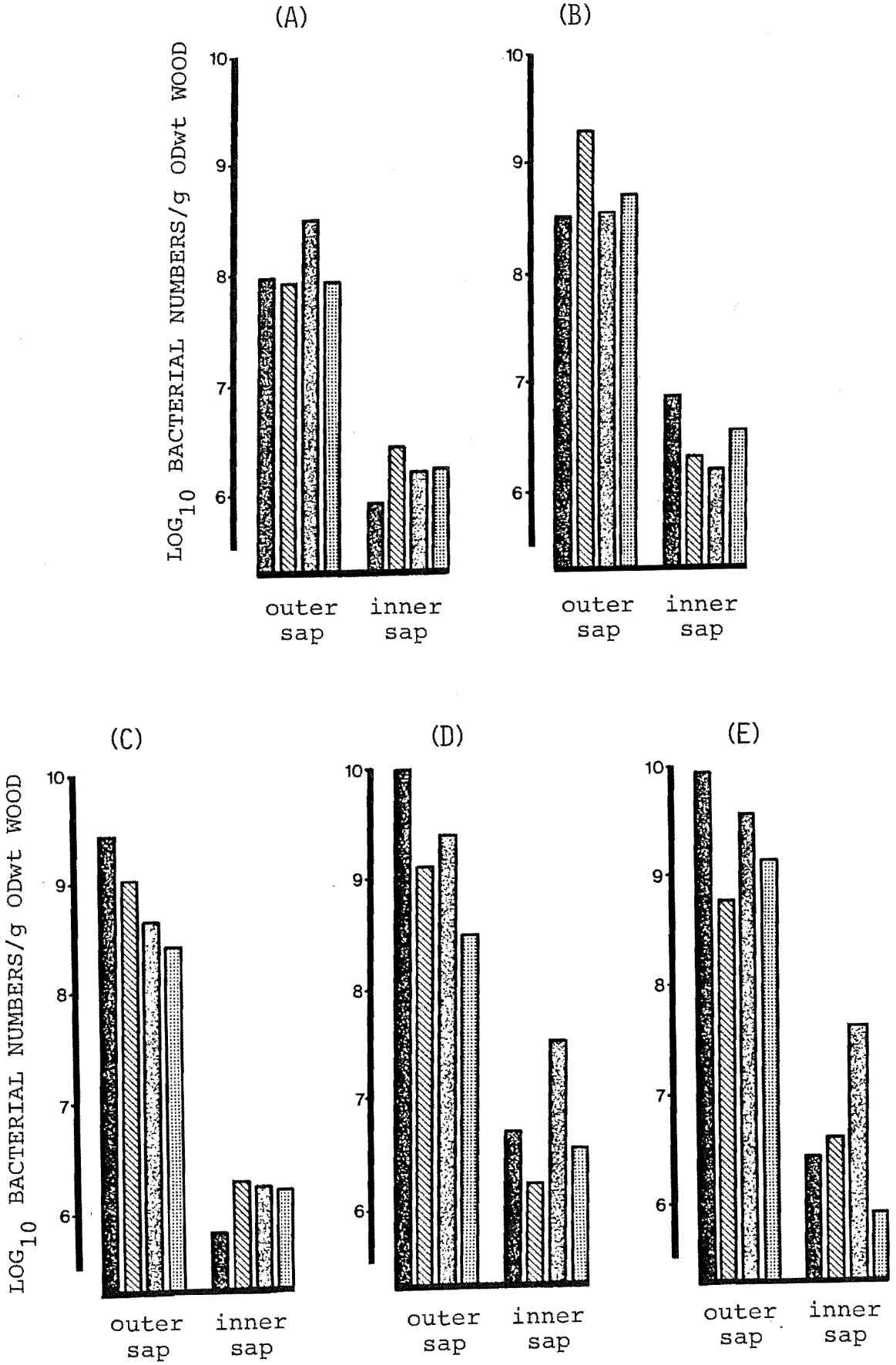
 Treatment 1 Buffer only

 Treatment 2 Nitrogen only

 Treatment 3 Nitrogen and Buffer

 Treatment 4 Water only

Figure 4-22


(A)
(B)
(C)
(D)
(E)
LOG10 BACTERIAL NUMBERS/g ODwt WOOD
10
9
8
7
6
outer sap
inner sap

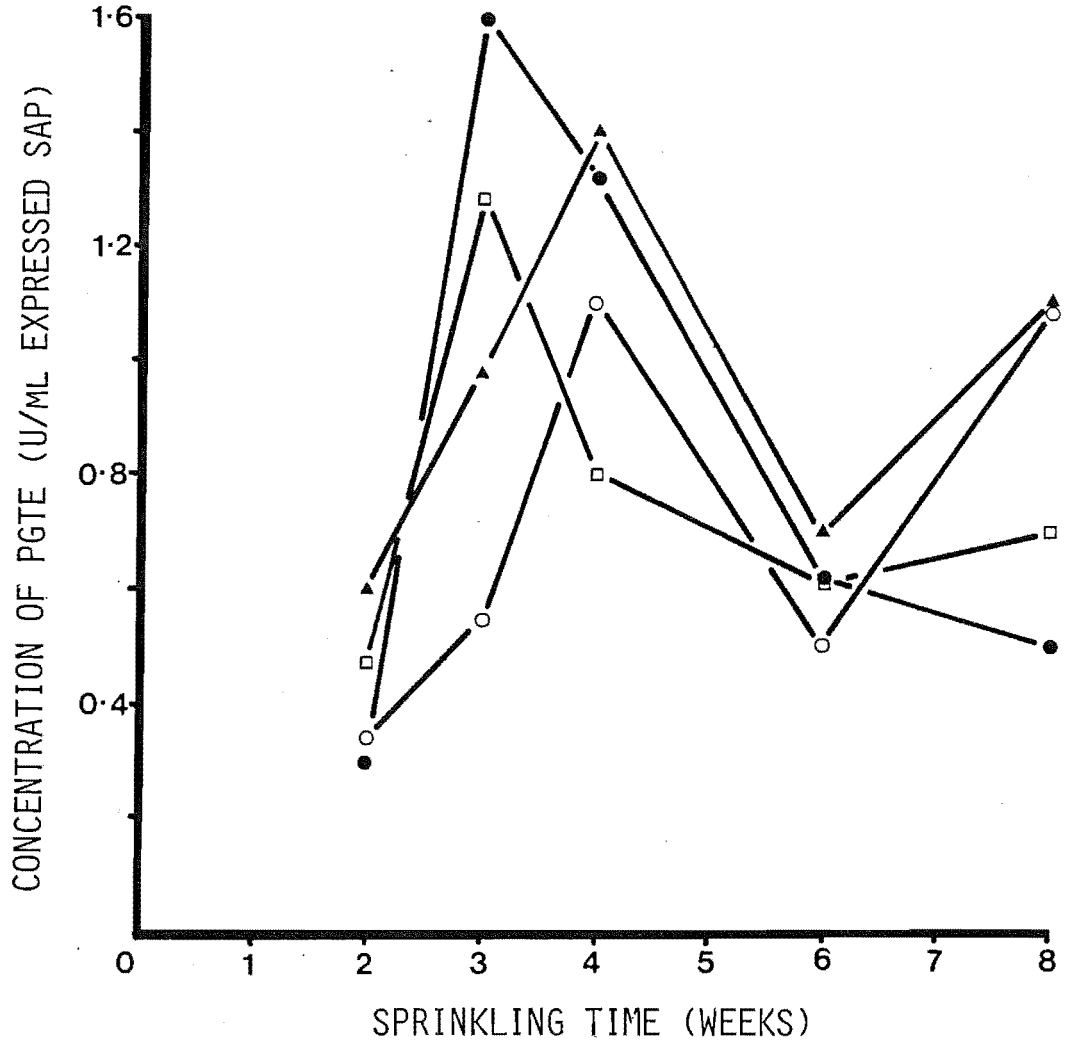
number of bacteria isolated from both kerfed and non-kerfed material was similar. It seems likely that the peak bacterial count, between 10^7 and 10^{10}, represents the maximum, sustainable population under the conditions present in sprinkled wood.

There was no change in the inner sapwood bacterial count until week 6 and then it occurred in treatment 3 only. That situation remained unchanged after eight weeks' sprinkling. There was no change in the size of the inner sapwood bacterial population in treatments 1, 2 and 4 at any sprinkling time suggesting that radial migration of bacteria into the wood was extremely slow.

4.5.5 PGTE concentration in non-kerfed wood

The concentration of PGTE in the outer sap of non-kerfed wood at different sprinkling times is shown in Figure 4-23. Comparing the data in Figure 4-23 with those in Figure 4-8 reveals that the peak PGTE concentration in non-kerfed wood was substantially lower than that in kerfed wood. The maximum PGTE concentration recorded for non-kerfed wood in any treatment was 1.6 U/ml of expressed sap, one quarter of the maximum occurring in kerfed wood. The result is surprising because it was expected that enzyme concentrations in both kerfed and non-kerfed bolts would be similar. It is difficult to visualise how the presence or absence of a kerf can influence enzyme activity in the outer sapwood zone. The low enzyme concentration may be explained by enzyme denaturation; extensive browning of the expressed sap occurred before the samples could be frozen (Section

Figure 4-23 Concentration of PGTE in the outer sap of non-kerfed Douglas fir at different sprinkling times.



KEY TO SYMBOLS:

- ● Treatment 1 Buffer only
- ○ Treatment 2 Nitrogen only
- ▲ Treatment 3 Nitrogen and Buffer
- □ Treatment 4 Water only

4.4.5). Assuming that the loss due to browning reactions was constant among treatments and sprinkling times, the data graphed in Figure 4-23 indicate that PGTE concentration peaked after three weeks' sprinkling in treatments 1 and 4 and after four weeks' sprinkling in treatments 2 and 3.

Table 4-8 shows the concentration of PGTE in the inner

TABLE 4.8 CONCENTRATION OF PGTE IN THE INNER AND OUTER SAP OF NON-KERFED WOOD AFTER 6 AND 8 WEEKS SPRINKLING

TREATMENT	CONCENTRATION OF PGTE (U/ml) OUTER SAP	INNER SAP
6 WEEKS T1	0.6	___
6 WEEKS T2	0.5	___
6 WEEKS T3	0.6	___
6 WEEKS T4	0.6	___
8 WEEKS T1	0.5	0.06
8 WEEKS T2	1.1	0.08
8 WEEKS T3	1.1	0.1
8 WEEKS T4	0.7	0.04

(___) INDICATES NO MEASURABLE ENZYME

and outer sap of non-kerfed bolts at six and eight weeks sprinkling. No measurable amounts of PGTE were found in the inner sap until the bolts had been sprinkled for at least six weeks. The amount of PGTE recorded in the inner sap for all treatments after eight weeks was extremely low (0.09 U/ml) and is comparable with the amount occurring at position 1 (Table 4-3, Section 4.4.5) after four weeks.

4.5.6 Preservative treatment of non-kerfed bolts

Preservative treatment of the non-kerfed material was plagued by frequent end-seal failure. Severe checking, which arose during kiln drying, also influenced preservative uptake. Consequently, mean preservative uptake and depth of preservative penetration were affected substantially. Raw data for the preservative treatments are presented in Appendix R13. At least three major end-seal failures are noted but others were suspected. The raw uptake data were compared in a three way ANOVA ignoring the end-seal failures and substituting missing data estimations. Analysis of the data without the missing data estimations was inappropriate for two reasons; firstly, the data were not normally distributed and secondly, there were large differences in the error variances between treatment factors. A summary of the ANOVA can be found in Table 4-9. The table shows that

TABLE 4-9 SUMMARY OF ANOVA FOR NON-KERFED UPTAKE DATA

SOURCE OF VARIATION	SS	DF	MS	F	
WITHIN BUFFER TREATMENT	4389.025	1	4389.025	3.565	N.S.
WITHIN NITROGEN TREATMENT	23863.225	1	23863.225	19.385	***
WITHIN SPRINKLING TIMES	218035.850	4	54508.962	44.281	***
B X N INTERACTION	8850.625	1	8850.625	7.189	*
B X T INTERACTION	27335.350	4	6833.837	5.551	***
N X T INTERACTION	34279.650	4	8569.912	6.962	***
B X N X T INTERACTION	29110.750	4	7277.687	5.912	***
ERROR	24619.500	20	1230.975		
TOTAL	370483.975	39	9499.589		

the effect of adding buffer was not significant at a probability of P=0.05. In contrast both nitrogen and sprinkling time had a significant effect on uptake.

However, it is not possible to make a definitive statement about the influence of buffer, nitrogen or sprinkling time on preservative uptake because as Table 4-9 shows there were significant interactions among the three factors.

Uptake data are illustrated in Figure 4-24. It is obvious that sprinkling treatments improved preservative uptake. There were no significant differences in preservative uptake among the four treatments up to four weeks' sprinkling. However, differences among the treatments become evident at week 6. Treatments 2 and 3 enhanced uptake most of all. The uptake for treatment 4 at week 8 was suspiciously high and the result was probably due to partial failure of the end-seal.

End-seal failure and checking contributed to the preservative distribution in wood and thus the total uptake was not due to radial penetration alone. Measurements of both minimum and maximum radial depth of penetration were made and the results are tabulated in Appendix R14. The two values differ considerably in some of the treatments. Minimum penetration figures are commonly used to evaluate the efficacy of a preservative treatment. Minimum preservative penetrations in non-kerfed bolts are plotted in Figure 4-25. The graph shows that the depth of penetration increased three-fold over the non-sprinkled material after two weeks' sprinkling. There were no apparent differences among the treatments at that stage. By week 3 the depth of penetration had increased further. Accurate measurements for treatments 3 and 4 at week 3 were affected by end-seal failure. The asterisk above those two points on the graph highlights the fact that they were obtained from one

Figure 4-24 Uptake of CCA preservative in non-kerfed Douglas fir at different sprinkling times

KEY TO SHADING:


Non-sprinkled


Treatment 1 Buffer only


Treatment 2 Nitrogen only


Treatment 3 Nitrogen and Buffer


Treatment 4 Water only

Least significant difference P=0.05

Figure 4-25 Radial penetration of CCA preservative into non-kerfed Douglas fir.

KEY TO SHADING: as above

Figure 4-24

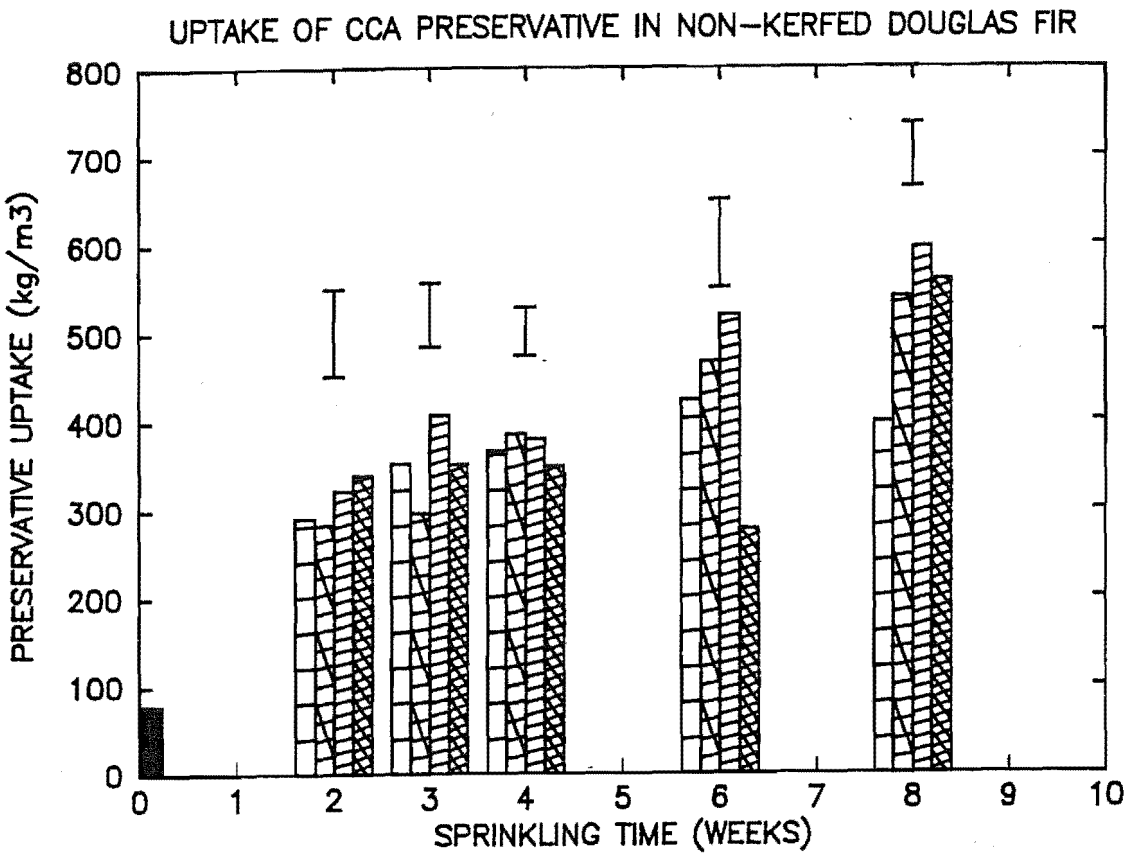
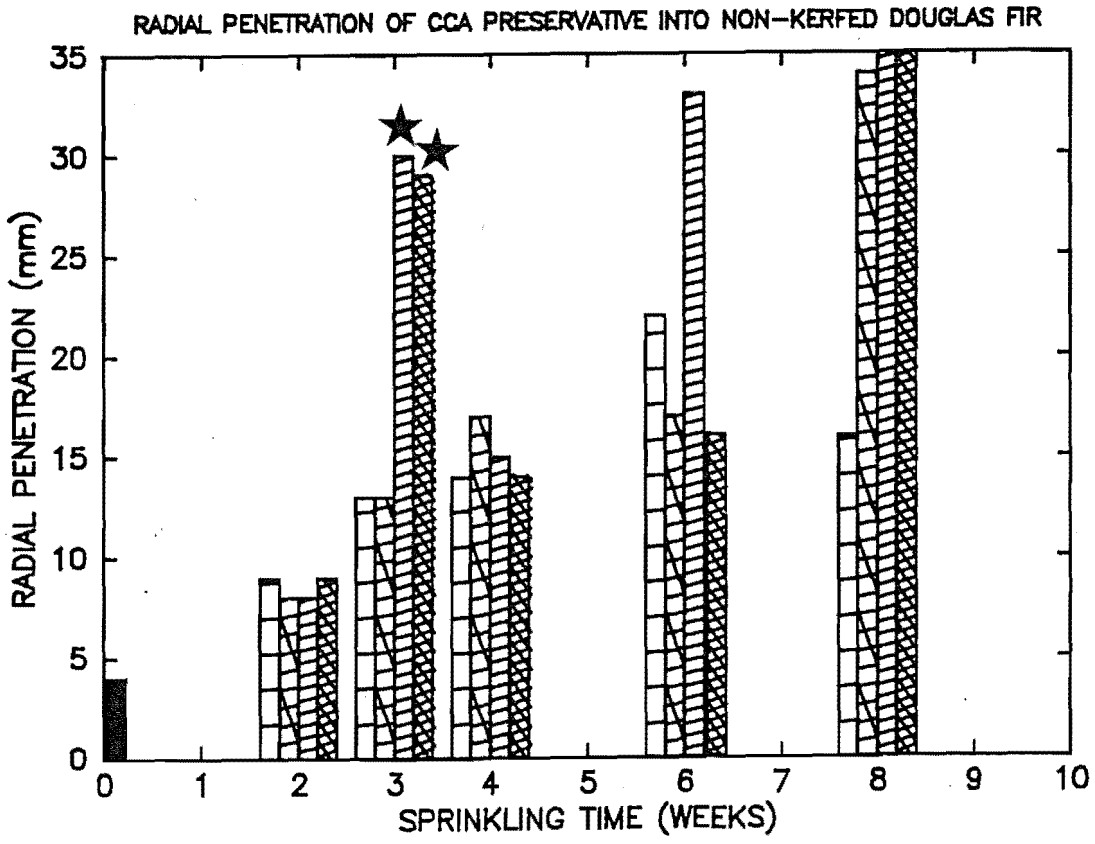


Figure 4-25



replicate only. There was no improvement in penetration from three to four weeks' sprinkling. By six weeks however, penetration had increased in all treatments and increased further by eight weeks' sprinkling. Total sapwood penetration was achieved in treatments 2, 3 and 4 at eight weeks' sprinkling. The exceptional result for treatment 4 was unexpected and should be treated with caution. The relatively poor penetration in bolts taken from treatment 1 was contrary to that expected on the basis of results from the kerfed bolts.

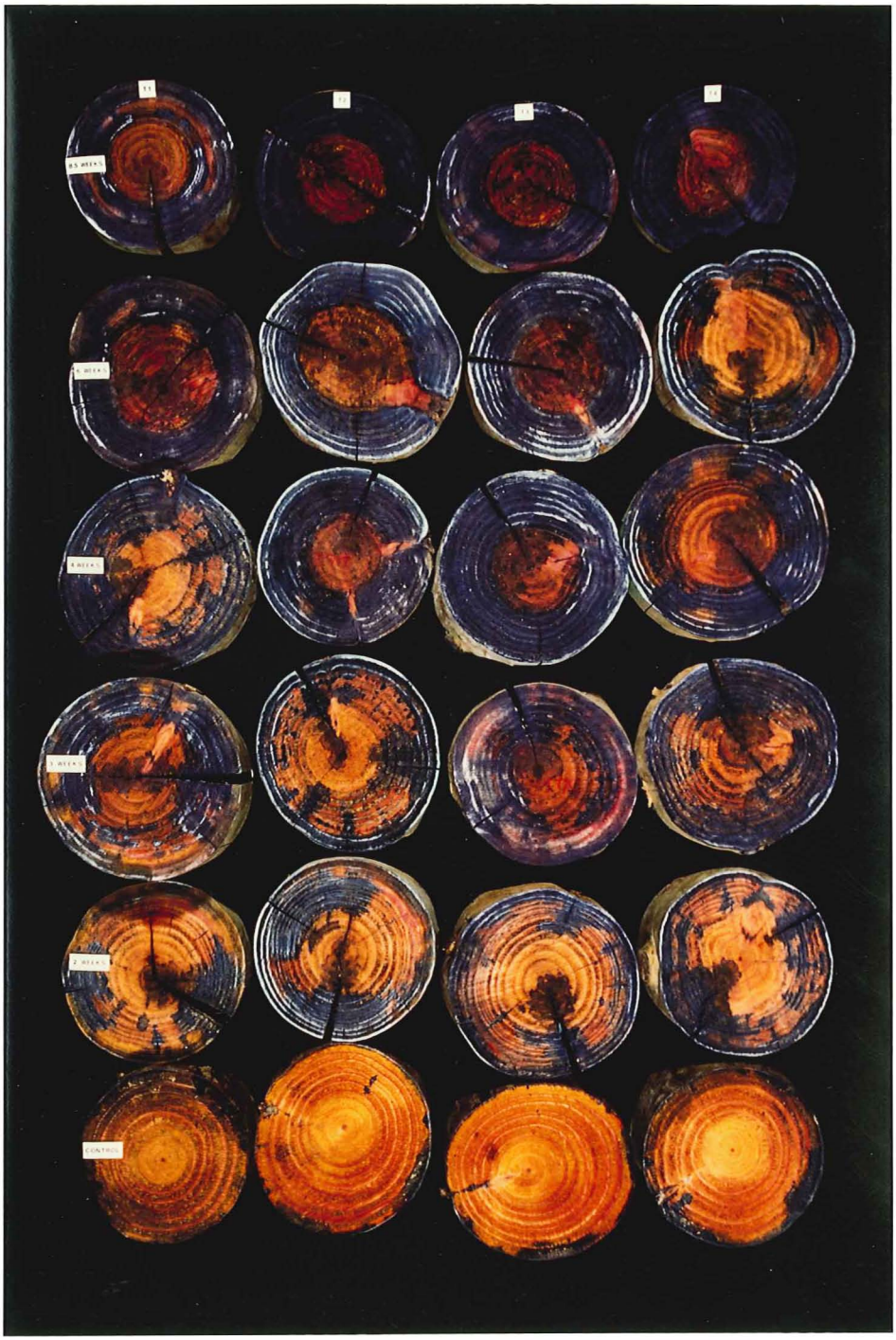
In view of such aberrant data it is difficult to make any further comment on the relative effectiveness of the four treatments. The adjusted data plotted in Figures 4-24 and 4-25 show that preservative uptake and radial depth of penetration increased with sprinkling time. Regression analysis to quantify those relationships did not seem appropriate with such a poor data set.

The variability of the preservative distribution in the non-kerfed bolts is illustrated in Figure 4-26. The contribution of tangential preservative penetration through the numerous checks is obvious from the photograph. The additional preservative uptake provided by the checks appeared beneficial but the preservative distribution around the checks was uneven and was largely confined to the latewood.

Preservative loadings in non-kerfed bolts sprinkled for eight weeks are presented in Table 4-10. The table shows that the outer and inner sapwood of all treatments contained an acceptable level of all three preservative components. It is perhaps worthy of note that the level of potassium in the

Figure 4-26 Photograph showing the penetration of CCA in non-kerfed Douglas fir at different sprinkling times.

Freshly cut faces sprayed with chrome-azurol. Blue colour is positive for copper.



- 8 weeks
- 6 weeks
- 4 weeks
- 3 weeks
- 1 week
- Control non-sprinkled

Buffer | Nitrogen | Nitrogen and Buffer | Water only

TABLE 4-10 LOADINGS OF INDIVIDUAL PRESERVATIVE COMPONENTS IN NON-KERFED DOUGLAS FIR SPRINKLED FOR 8 WEEKS

SPRINKLING TREATMENT	POSITION	ELEMENTAL LOADING (% O.D. WT.) Cu	Cr	As	TOTAL ACTIVE ELEMENTS (%)	POTASSIUM LEVEL (KCPS)
1	OUTER SAP	0.40	0.49	0.57	1.46	2.279
	INNER SAP	0.47	0.63	0.66	1.76	1.301
2	OUTER SAP	0.46	0.54	0.63	1.63	
	INNER SAP	0.50	0.58	0.67	1.75	
3	OUTER SAP	0.37	0.44	0.50	1.31	2.242
	INNER SAP	0.37	0.37	0.51	1.25	1.093
4	OUTER SAP	0.40	0.46	0.56	1.42	
	INNER SAP	0.41	0.40	0.55	1.36	

outer sap of treatments 1 and 3 was twice that of the inner sap. The high potassium levels associated with the lower copper and chrome levels in the outer sap of treatment 1 confirms the results obtained with kerfed bolts (Section 4.4.6). That does not seem to be the case with the treatment 3 data and it suggests that the result was probably spurious.

4.6 MICROSCOPIC EVIDENCE OF BACTERIAL ATTACK

Bacterial colonisation of Douglas fir is a dynamic process and can be extensive. When examining the effects of bacteria in sprinkled wood it is important to consider the duration of exposure. Further not all areas of a round bolt are colonised at the same time. Thus the samples for

examination should come from roughly equivalent areas in different bolts so that valid comparisons can be made.

The light microscope and the scanning electron microscope (SEM) were used to examine samples of sprinkled wood for evidence of bacterial attack. The photographs which follow are entirely confined to earlywood tissues for two reasons:

> [1] Preparation artifacts were prevalent in latewood tissues. It was difficult to cut a clean surface using a razor blade and freeze drying often resulted in extensive cracking of the tissues
>
> [2] Earlywood tissues are a greater hindrance to preservative uptake when dried and therefore the effects of bacteria on those tissues seemed most important

The light microscope was useful in establishing the distribution of bacteria within the wood. Figure 4-27(a) shows a normal bordered pit from a non-sprinkled bolt after staining with ruthenium red. Note the accumulation of stain in the torus. By comparison, after one week of sprinkling, bacteria were found clustered in the bordered pit chambers (Figure 4-27(b)) and also occupying the tracheid walls in the vicinity of the tracheid to ray pits. The resolution of the light microscope was not sufficient to discern any changes in the pit membrane which could be related to the presence of bacteria, although a decrease in the staining intensity of the torus was common. That observation was purely subjective and impossible to quantify. There was no evidence of bacteria within the ray tissues.

The superior resolution of the scanning electron microscope makes it possible to detect changes in wood

Figure 4-27

A Light micrograph showing a typical sapwood, earlywood bordered pit. Wet mount stained with ruthenium red. Scale bar = 5 µm

B Light micrograph showing bacteria clustered in an earlywood bordered pit chamber after one week sprinkling. Wet mount stained with ruthenium red. Scale bar = 5 µm

C Scanning electron micrograph of normal earlywood bordered pit. Freeze-dried preparation gold coated. Scale bar = 5 µm

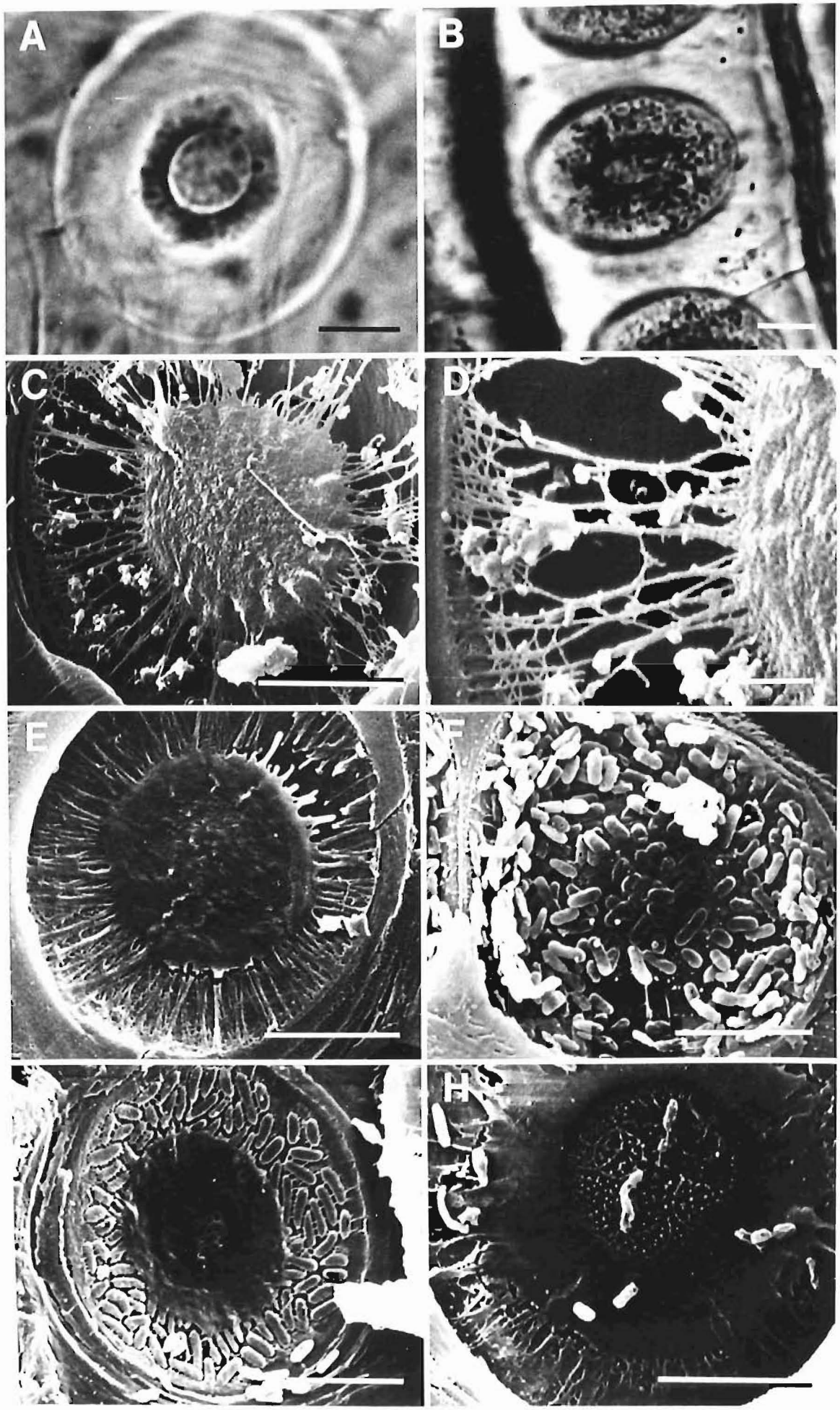
D Higher magnification of (C) showing detail of margo fibrils. Scale bar = 1 µm

E Scanning electron micrograph of a partially aspirated earlywood bordered pit. Specimen air-dried coated with gold. Scale bar = 5 µm

F Scanning electron micrograph showing extensive bacterial colonisation of a bordered pit membrane and pit chamber after two days sprinkling. The upper pit border has been removed during preparation. Freeze-dried preparation gold coated. Scale bar = 5 µm

G Scanning electron micrograph showing an earlywood bordered pit colonised by bacteria. Note the dense clustering of bacterial cells on the margo and small perforation in the torus. Freeze-dried preparation coated with gold. Scale bar = 5 µm

H Scanning electron micrograph showing early stage of torus degradation. Note the granular region in the centre of the torus. Freeze-dried preparation coated with gold. Scale bar = 5 µm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structure. A typical non-aspirated bordered pit from a non-sprinkled bolt, freeze dried from the green state, is shown in Figure 4-27(c). A higher magnification of the same pit showing detail of the torus and margo is provided in Figure 4-27(d). These structures are extremely fragile and even though great care was taken during specimen preparation damage to the margo seemed unavoidable. However, Figure 4-27(c) and 4-27(d) show clearly the impervious nature of the torus and the open-structured, fibrillar margo. It was more common, even in freeze dried preparations, to see partially aspirated bordered pits (Figure 4-27(e)). The torus was displaced to one side of the pit, effectively sealing the pit aperture.

After sprinkling for only 1-2 days, bordered pits between tracheids close to the cambial surface of non-kerfed bolts and close to the kerf in kerfed bolts were colonised by large numbers of bacteria (Figure 4-27(f) and 4-27(g)). A short time later the center of the torus became granular. Typically the granular area was circular and of similar dimension to the under-lying pit aperture (Figure 4-27(h)). No changes in the margo were visible at that stage. The torus became more granular (Figure 4-28(a)) and ultimately small holes appeared. This resulted in the formation of a 'lacy' network (Figure 4-28(b)). Higher magnification of the 'lacy' structure reveals its fibrillar nature, similar to that of the margo (Figure 4-28(c)). Eventually the small holes making up the network seemed to coalesce, producing a single central opening (Figure 4-28(d) and 4-28(e)) giving the torus a 'doughnut' appearance. Note that in both these photographs the margo is still

Figure 4-28

A Scanning electron micrograph showing further development of the granular degradation pattern on an earlywood bordered pit torus. Note the presence of numerous rod-shaped bacteria and the lack of degradation of the margo. Freeze-dried preparation gold coated. Scale bar = 5 µm.

B Scanning electron micrograph of a bordered pit torus viewed through the pit aperture showing a 'lacy' appearance after removal of the pectinaceous matrix materials by bacterial enzymes. Freeze-dried preparation gold coated. Scale bar = 5 µm

C Scanning electron micrograph showing the fibrillar nature of the 'lacy' torus. Freeze-dried gold/ platinum coated. Scale bar = 1 µm

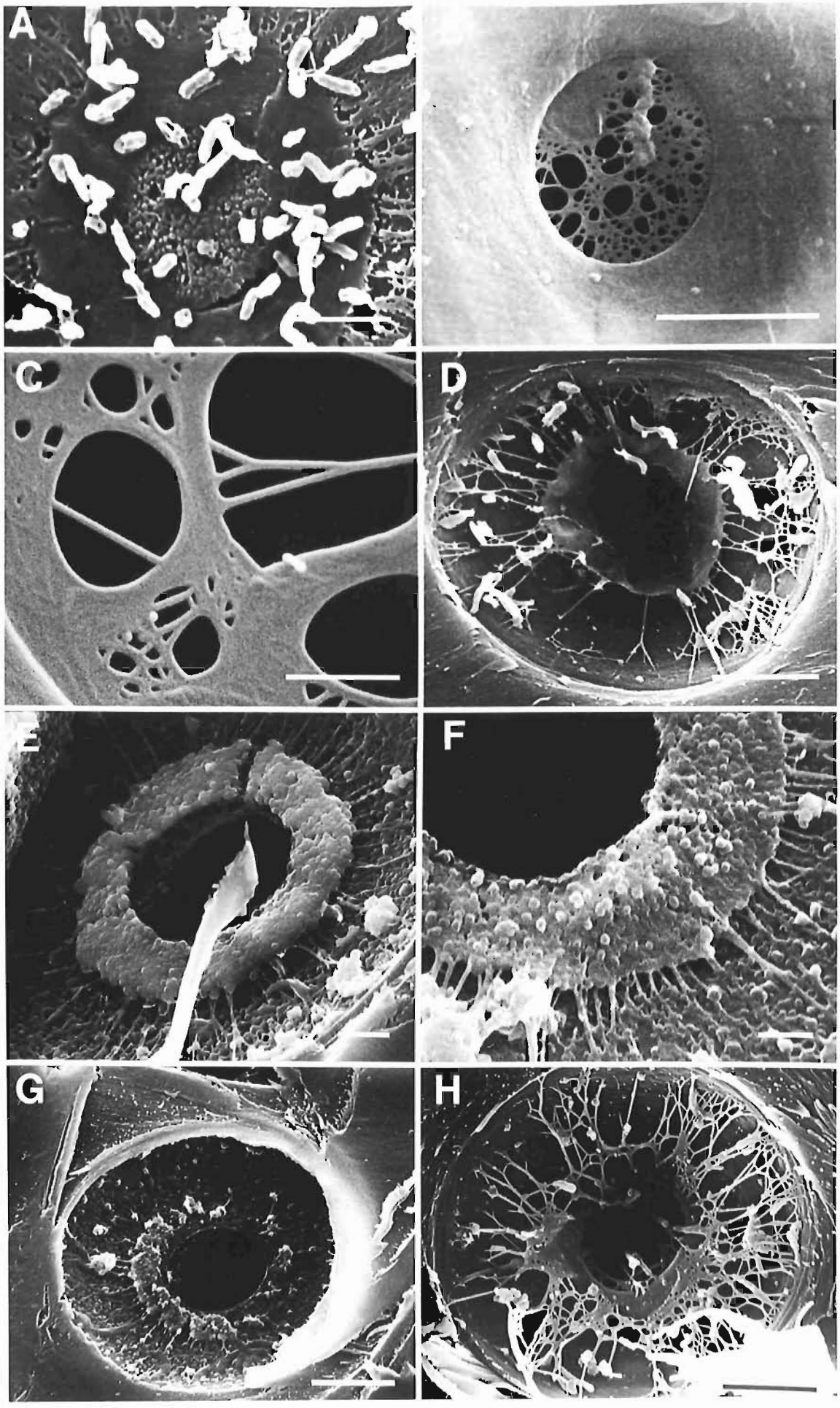
D Scanning electron micrograph showing complete disappearance of the central torus region. Note the presence of several rod-shaped bacteria adhering to the relatively intact margo fibrils. Freeze-dried Au/Pt coated. Scale bar = 5 µm

E Scanning electron micrograph showing a similar stage of degradation to (D). Note the doughnut appearance of the remaining portions of the torus, the intact margo and the granular material encrusting the pit chamber and the torus. Freeze-dried Au/Pt coated. Scale bar = 1 µm

F Scanning electron micrograph showing a degraded torus at higher magnification. Note the granular appearance of the surface of the remaining portions of the torus and the surface of the pit chamber. The hole present in the torus has a relatively smooth outline. Freeze-dried preparation Au/Pt coated. Scale bar = 5 µm

G Scanning electron micrograph showing partial degradation of the remaining 'ring' of torus. Note that at this late stage of pit destruction some margo fibrils remain intact. Freeze-dried preparation Au/Pt coated. Scale bar = 5 µm

H Scanning electron micrograph showing almost complete loss of the torus but the margo remains relatively intact. Freeze-dried preparation Au/Pt coated. Scale bar = 5 µm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present. At higher magnifications the surface of the annular remains of the torus have a granular appearance while the edge of the hole has a relatively smooth outline (Figure 4-28(f)). Figure 4-28(g) shows what is thought to be a later stage in the destruction of the torus when the remaining 'ring' had been partially degraded. Even at this late stage the margo was still present. This is more noticeable in Figure 4-28(h) in which the membrane is not so aspirated. Ultimately all trace of the torus disappeared leaving the unsupported margo strands lining the pit chamber. The strands were extremely fragile and apparently elastic. Frequently the bordered pit chambers appeared totally devoid of torus and margo. Finally the majority of all bordered pits in the tracheids were removed (Figure 4-29(a)).

The fate of the margo poses an interesting problem. It is difficult to decide whether it was degraded by the bacteria, removed during specimen preparation or damaged by the electron beam once the torus had been lost. Frequently margo fibrils remaining within a pit chamber retracted under the electron beam, implying that their disappearance is more likely to be an artifact. However, the margo was subject to enzyme attack. A bordered pit after incubation with a commercial hemicellulase and polygalacturonase for 24h is shown in Figure 4-29(b). It is interesting to observe the extensive degradation of the margo and the partial degradation of the torus.

Figure 4-29(c) shows the typical appearance of the piceoid, tracheid-to-ray crossfield pits from a non-sprinkled sample, viewed from the tracheid side. Similar pits viewed from the ray side can be seen in Figure 4-29(d)

Figure 4-29

A Scanning electron micrograph showing a field of degraded bordered pits. Freeze-dried preparation gold/platinum coated. Scale bar = 10 µm

B Scanning electron micrograph of bordered pit treated with commercial pectinase and hemicellulase for 24h. Note the extensive degradation of the margo and partial attack of the torus. Air dried preparation gold coated. Scale bar = 5 µm

C Scanning electron micrograph showing the typical appearance of the piceoid tracheid-to-ray crossfield pits viewed from the tracheid side. Freeze-dried preparation Au/Pt coated. Scale bar = 5 µm

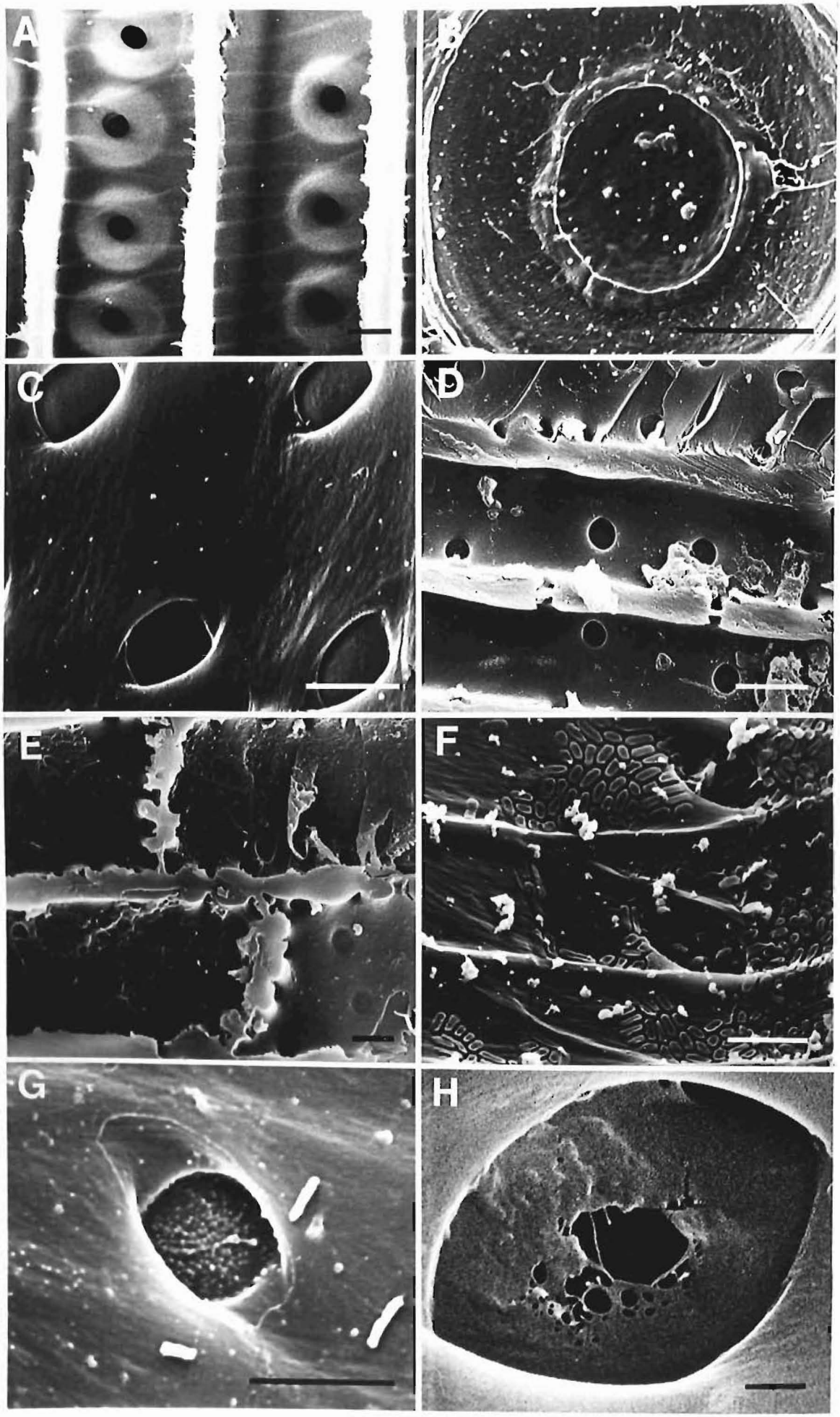
D Scanning electron micrograph showing tracheid-to-ray pits viewed from the ray side. The same photograph also shows parenchyma-parenchyma simple pits in cross section. Freeze-dried preparation Au/Pt coated. Scale bar = 10 µm

E Scanning electron micrograph showing tracheid-to-ray and simple pits on the end walls of parenchyma cells. Note also the amorphous coating covering the inner walls of the parenchyma cells. Freeze-dried preparation Au/Pt coated. Scale bar = 10 µm

F Scanning electron micrograph showing large numbers of bacteria adhered to a tracheid wall in the vicinity of a tracheid-to-ray pit field after three days sprinkling. Freeze-dried preparation Au/Pt coated. Scale bar = 5 µm

G Scanning electron micrograph showing an early stage in the degradation of a tracheid-to-ray pit membrane. Note the granular appearance of the membrane viewed from the tracheid side. Freeze dried preparation Au/Pt coated. Scale bar = 5 µm

H Scanning electron micrograph showing a later stage in the degradation sequence of a tracheid-to-ray pit membrane. The granular regions develop into holes and the centre of the membrane becomes 'lacy', similar to that seen earlier with bordered pits in Figure 4-28(b). Freeze-dried preparation Au/Pt coated. Scale bar = 1 µm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and 4-29(e). The lack of any visible openings in the membranes of these pits contrasts sharply with the bordered pits in Figure 4-27(c) and 4-27(d). The internal appearance of the ray cell walls and pit membranes in Figure 4-29(d) is atypical. Usually the walls were obscured by a coating of cell debris (Figure 4-29(e)) (arrowed). A cross-sectional view of parenchyma—to—parenchyma simple pitting is also evident on the end and side walls of ray cells (Figure 4-29(d) and 4-29(e)). Visible pores are absent in these pit membranes also.

The degradation of the tracheid-to-ray pits follows a sequential process similar to that of the bordered pits, although it took longer. Bacterial colonisation of the tracheid walls in the vicinity of a field of tracheid-to-ray pits could be quite extensive. Figure 4-29(f) shows the presence of large numbers of bacteria after a three day sprinkling period. Early in the colonisation process bacteria had no apparent effect on the tracheid-to-ray pits while the adjacent bordered pits could be totally destroyed. In other words bordered pits were degraded before tracheid-to-ray pits. Eventually the tracheid-to-ray pit membranes became granular (Figure 4-29(g)). The granules developed into holes (Figure 4-29(h), 4-30(a)) and ultimately the membranes were completely perforated (Figure 4-30(b)). Few bacteria were found colonising the interior of the ray parenchyma cells within the time span of the sprinkling treatments, suggesting that tracheid-to-ray pits were attacked from the tracheid side. As a consequence, no degradation of the parenchyma-to-parenchyma simple pits was observed. Figure 4-30(c) shows perforated tracheid-to-ray

Figure 4-30

A Scanning electron micrograph showing partially degraded tracheid-to-ray pit from the ray cell side. Although the specimen is extensively beam damaged, it is possible to see the 'lacy' appearance of the membrane. Freeze-dried preparation Au/Pt coated. Scale bar = 1 µm

B Scanning electron micrograph showing several tracheid-to-ray pit membranes in adjacent parenchyma cells completely removed after eight weeks sprinkling. Freeze-dried preparation Au/Pt coated. Scale bar = 10 µ m

C Scanning electron micrograph showing completely undegraded simple pits on the end wall of a ray parenchyma cell. Note the 'lacy' appearance of the adjacent tracheid-to-ray pits indicating a difference in ease of degradation between the two pit types. Freeze-dried preparation Au/Pt coated. Scale bar = 5 µm

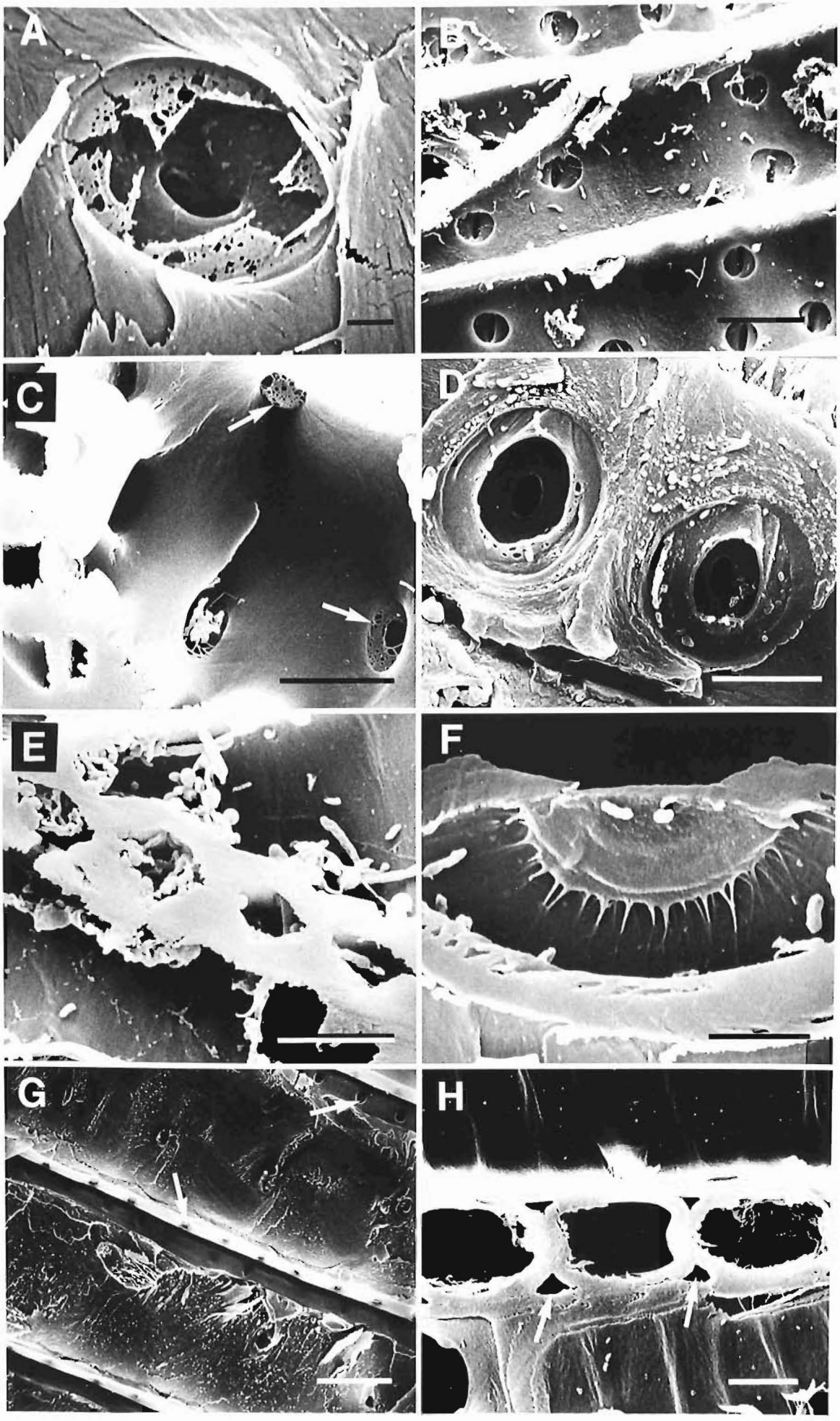
D Scanning electron micrograph showing the degradation of ray tracheid bordered pits. Freeze-dried preparation Au/Pt coated. Scale bar = 5 µm

E Scanning electron micrograph showing ray tracheid bordered pits on the end walls of a ray tracheid cell in cross section. Note the presence of bacteria both inside and outside of the pit chambers. Freeze-dried preparation Au/Pt coated. Scale bar = 5 µm

F Scanning electron micrograph showing a heartwood bordered pit after eight weeks sprinkling. Note the presence of bacteria and the absence of any visible degradation. Freeze-dried preparation Au/Pt coated. Scale bar = 5 µm

G Scanning electron micrograph showing the prevalence of 'blind' pits (arrowed) connecting interstitial spaces with ray parenchyma. Freeze dried preparation Au/Pt coated. Scale bar = 10 µ m

H Scanning electron micrograph showing tangential longitudinal section through a ray. Note the presence of interstitial spaces in the corners of the ray cells (arrowed). Freeze-dried preparation Au/Pt coated. Scale bar = 10 µm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H

pits (arrowed) with no visible degradation in the adjacent parenchyma-to-parenchyma simple pits.

Ray tracheids and their associated bordered pits were rarely encountered in the specimens prepared for examination. However, when found, extensive degradation of the bordered pit membranes was evident (Figure 4-30(d)). Typically, ray tracheid bordered pits lacked the well defined torus of their axial counterparts. However, degradation seemed to be confined to the middle of the membrane as before. Tori were present in bordered pits on the end walls of ray tracheid cells, but they were never seen in plan view. Figure 4-30(e) illustrates colonisation of the bordered pit chambers between two ray tracheid cells. A degradation pattern similar to that of the axial tracheid bordered pits probably occurred.

Several heartwood samples were examined for bacterial degradation even though bacterial colonisation was rare. Figure 4-30(f) shows a typical aspirated heartwood bordered pit with a few bacteria present. No degradation of the torus is visible.

Numerous 'blind' pits were observed between ray parenchyma and interstitial spaces (Figure 4-30(g)), but there was no evidence to indicate that the 'blind' pits were degraded by bacteria within an eight week sprinkling period. Interstitial spaces in ray tissues were quite common (Figure 4-30(h)) and were thought to be potentially capable of conducting fluids radially.

In the early stages of sprinkling, after about two weeks, debris coating cell walls and occluding pit membranes was frequently observed in samples of earlywood. Two forms

of debris were observed, one fibrous in nature (Figure 4-31(a) and the other granular (Figure 4-31(b)). It is probable that the two forms have the same composition and that the visual differences are preparation artifacts. Granular debris was most commonly observed with poorly freeze dried specimens. The fibrous form was generally associated with successfully freeze dried specimens. The cell surfaces of air dried specimens often appeared to be coated with a featureless 'film' of debris. In some instances this 'film' was sufficient to disguise the underlying wall structure, for example the helical thickening on the tracheid walls.

The ability of the debris to occlude pit membranes is obvious in Figure 4-31(c). This photograph shows a tracheid-to-ray pit membrane from the ray side, covered with a fibrous web of slime(?). At first glance such a web may be mistaken for a degraded pit membrane. However, because the material extends from the pit membrane, through the pit aperture to the lumen surface, this is unlikely. The existence of a coating is even more obvious in Figure 4-31(d). Again this photograph shows the interior of a ray cell and a tracheid-to-ray pit. A fibrillar web is present coating the cell but more interesting is the layer of material covering most of a pit membrane (arrowed). In many cases it was difficult to differentiate between a coating on the membrane and degradation of the membrane itself. Such a case is shown in Figure 4-31(e). The distinction in bordered pits is even more difficult (Figure 4-31(f)) because a fibrillar stage occurred in the normal sequence of events leading to the degradation of the torus. Figure 4-31(f)

Figure 4-31

A Scanning electron micrograph showing fibrous 'slime' occluding the pit aperture of an earlywood bordered pit and coating the tracheid wall. Freeze-dried preparation Au/Pt coated. Scale bar = 10 µm

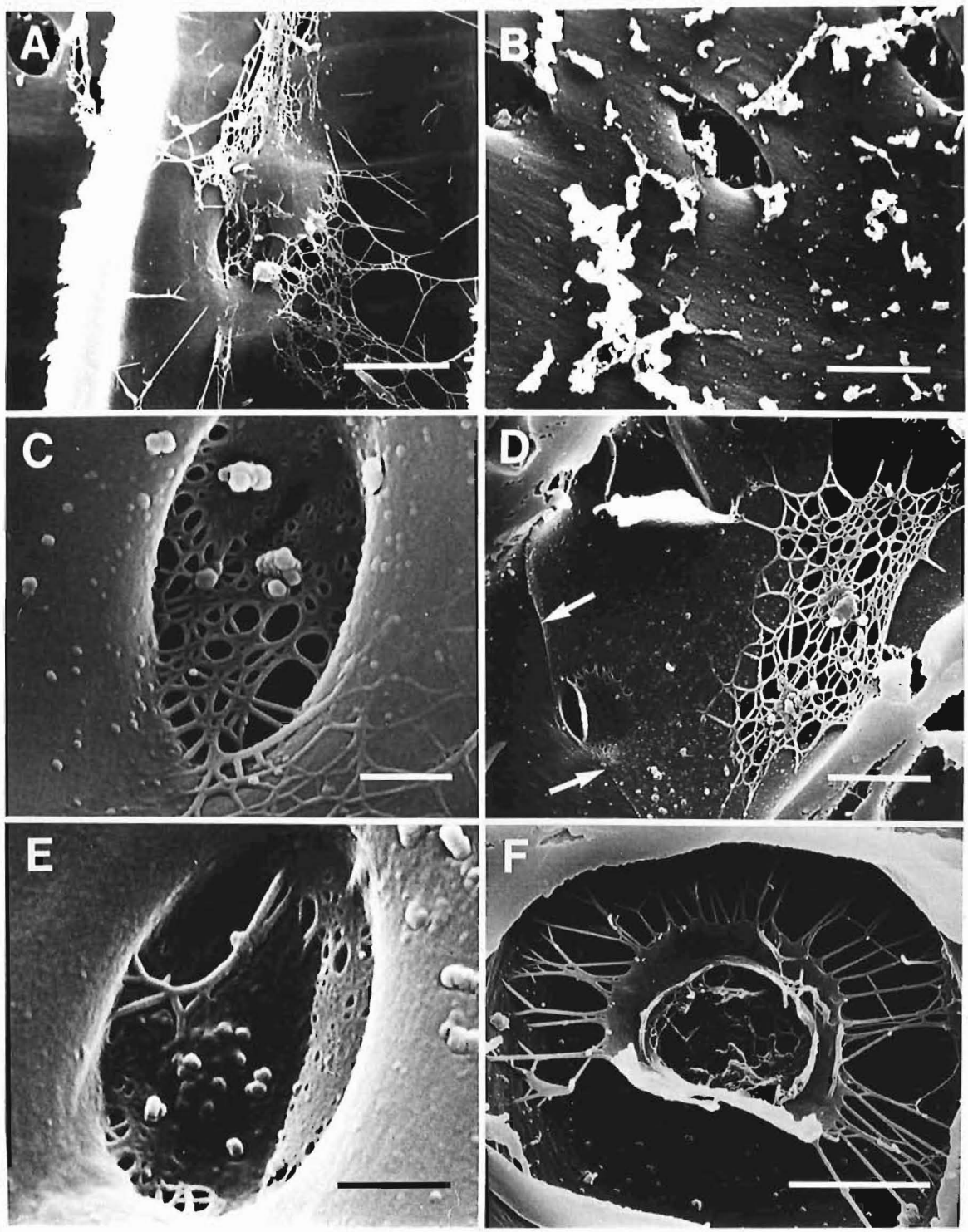
B Scanning electron micrograph showing the granular appearance of debris on an earlywood tracheid wall adjacent to a tracheid-to-ray pit. Air-dried preparation Au/Pt coated. Scale bar = 5 µm

C Scanning electron micrograph showing a tracheid-to-ray pit membrane viewed from the tracheid side covered with a web a fibrillar material. Note the similarity of the web to a degraded pit membrane. Freeze-dried preparation Au/Pt coated. Scale bar = 1 µm

D Scanning electron micrograph showing a ray cell viewed from inside. Note the existence of fibrillar material and an almost amorphous coating covering a tracheid-to-ray pit (arrowed). Freeze-dried preparation Au/Pt coated. Scale bar = 5 µm

E Scanning electron micrograph showing similar pit membrane to (C). Note the difficulty in determining whether or not fibrillar 'slime' material is present. Freeze-dried preparation Au/Pt coated. Scale bar = 1 µm

F Scanning electron micrograph showing a partially degraded bordered pit. Fibrillar material occluding the hole in the torus disappears into the background. Freeze-dried preparation Au/Pt coated. Scale bar = 5 µm


A
B
C
D
E
F

shows a bordered pit with a partially degraded torus. Fibrillar material is present (in the background) disappearing through the pit aperture into the lumen.

4.7 MISCELLANEOUS OBSERVATIONS

4.7.1 Discolouration of sprinkled wood

One consequence of prolonged sprinkling was a mild surface discolouration of the sprinkled bolts. Bolts sprinkled for 7-8 weeks generally turned a tan to light brown colour, particularly on the upper surfaces exposed to the constant spray. The undersides of sprinkled bolts, protected from the direct spray, were generally found to be covered with slime, which when removed, revealed normally coloured wood. The discolouration was not considered important because it was confined to the surface.

A reddish-brown discolouration developed on the exposed cross-cut ends of sprinkled bolts as they dried. The discolouration did not appear on the non-sprinkled material or on material sprinkled for less than three weeks and was confined to the surface only. In non-kerfed bolts the discolouration occurred as a continuous ring around the circumference. The width of that ring increased with time but reached an apparent maximum after six weeks. A similar coloured ring developed on kerfed material, although in that case it was accompanied by staining of the inner sap next to the kerf. The amount of discoloured inner sapwood increased with time. Cross-sections of non-kerfed bolts showing the reddish-brown discolouration are illustrated in Figure

4-32. The wood was sprinkled for six weeks. The photograph

Figure 4-32 Photograph of cross-cut ends of kiln dried Douglas fir after eight weeks sprinkling.
Note the brown discolouration of the outer sapwood and surface colonisation of the inner sapwood by fungi.

shows that the surfaces of the bolts had been colonised by fungi and that the colonisation was limited to the inner sapwood. The sharp delineation seen in the photograph suggests that fungitoxic compounds may have been present in the coloured area.

4.7.2 Measurement of permeability with a permeability cell

Preservative uptake can be regarded as an indirect measurement of the permeability of dried wood. It was of interest to know directly how the sprinkling treatment was affecting the permeability of 'green' wood. Such knowledge would help to clarify whether the reduction in preservative uptake after 2-4 weeks sprinkling was due to changes occurring before or after drying of the wood.

Measurements of permeability were made using a permeability cell in accordance to the methods of Booker 1977 and Booker 1980 (a) and (b) unpublished. The axial, radial and tangential permeabilities of samples cut from two week sprinkled, kerfed Douglas fir bolts were compared with corresponding samples cut from non-sprinkled bolts. Details of the sampling procedure used can be found in Figure 4-33. Results are presented in Table 4-11.

Time constraints made it impossible to analyse a large number of replicate samples, but nevertheless the results serve to show orders of magnitude for the respective permeabilities. Table 4-11 shows that there was little difference in the axial permeability values of outer sapwood samples removed from sprinkled and non-sprinkled wood. It is also interesting to note that the axial permeability of inner sapwood was approximately half that of the outer sapwood.

Radial permeability was about 2 X 10 times lower than axial permeability. No obvious differences could be seen in the radial permeability values between sprinkled and

TABLE 4-11 AXIAL, RADIAL AND TANGENTIAL PERMEABILITY DATA FOR 'GREEN', SPRINKLED AND NON-SPRINKLED DOUGLAS FIR

AXIAL PERMEABILITY DATA

SPRINKLED BOLTS

POSITION	SAMPLE ID	PERMEABILITY 'K' X 10-12 m2
OUTER SAP NEXT TO KERF	S 1	6.96
	S 2	6.78
OUTER SAP OPPOSITE KERF	S 3	6.70
	S 7	7.47
INNER SAP NEXT TO KERF	S 9	3.76
	S 6	3.16
INNER SAP OPPOSITE KERF	S 10	3.75
	S 8	3.68

NON SPRINKLED BOLTS

POSITION	SAMPLE ID	PERMEABILITY 'K' X 10-12 m2
OUTER SAP	L 1	5.19
	L 3	6.74
INNER SAP	L 5	5.10

RADIAL PERMEABILITY DATA

SPRINKLED BOLTS

POSITION	SAMPLE ID	PERMEABILITY 'K' X 10-16 m2
CLOSE TO CAMBIUM	R 1	2.03
	R 2	3.09
	R 3	3.20
	R 4	2.23
INNER SAP	R 5	2.38

NON-SPRINKLED BOLTS

POSITION	SAMPLE ID	PERMEABILITY 'K' X 10-16 m2
CLOSE TO CAMBIUM	CR 1	1.75
	CR 2	2.65
	CR 3	3.33
	CR 4	3.83
INNER SAP	CR 5	3.26

TANGENTIAL PERMEABILITY DATA

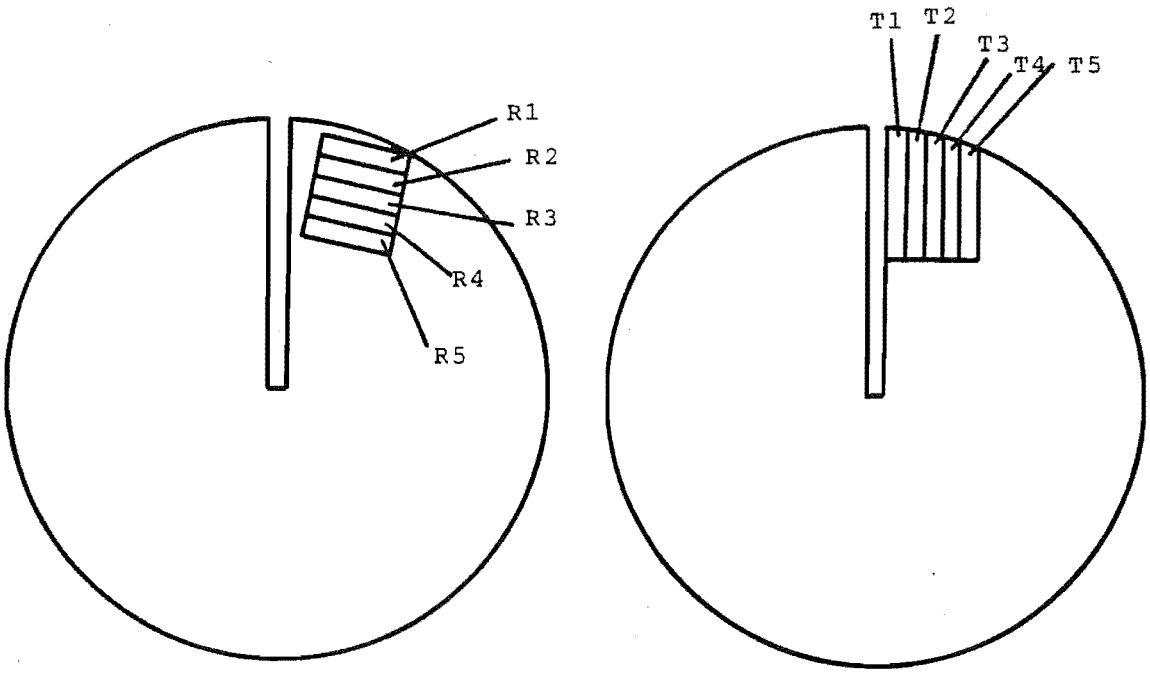
SPRINKLED BOLTS

POSITION	SAMPLE ID	PERMEABILITY 'K' X 10-16 m2
CLOSE TO KERF	T 1	5.13
	T 2	10.20
	T 3	13.60
FURTHEST FROM KERF	T 4	14.00

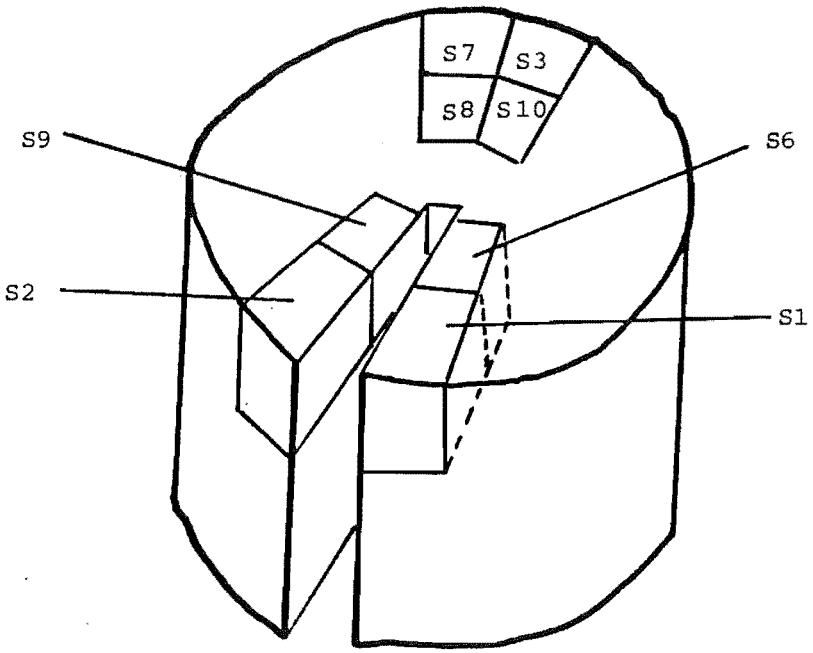
NON-SPRINKLED BOLTS

POSITION	SAMPLE ID	PERMEABILITY 'K' X 10-16 m2
	CT 1	11.21
	CT 2	9.95
	CT 3	13.80
	CT 4	15.80

Figure 4-33 Schematic diagram showing the sampling regions used for the permeability measurements presented in Table 4-11.



Radial permeability Tangential permeability



Axial permeability

NOTE: The sample labels apply to sprinkled bolts. For non-sprinkled bolts:

S1 = L1, S2 = L3, S6 = L5,

T1 = CT1, T2 = CT2, etc.

R1 = CR1, R2 = CR2, etc.

non-sprinkled material. Radial permeability in the outer sapwood was similar to that in the inner sapwood.

In contrast, two weeks' sprinkling appeared to have a negative influence on tangential permeability. Assuming that the tangential permeability of non-sprinkled material was typical of 'normal' Douglas fir then the tangential permeability of sample 'T1' removed from a position adjacent to the kerf was half that of the 'normal' value. The effect appears to be extremely localised because the tangential permeability of the adjacent sample was of similar magnitude to the non-sprinkled material. Because the area next to the kerf was colonised by bacteria first it seems plausible that the reduced tangential permeability was caused by bacterial cells which were able to block the pit membrane pores.

Although the results cannot be quantified statistically, the indications are that the tangential permeability of 'green' two week sprinkled Douglas fir was reduced. Axial and radial permeability appeared to be unaffected after sprinkling for two weeks.

CHAPTER 5

DRYING AND SHRINKAGE OF DOUGLAS FIR AFTER WATER SPRINKLING

5.1 GENERAL INTRODUCTION

Timber must be dried before it can be pressure impregnated with a preservative using Bethel process. Typically, wood is dried to approximately fibre saturation point so that the void spaces are free of liquid and the greatest possible preservative penetration and retention can be achieved. While Douglas fir air dries readily, free from major defects such as severe checking and honeycombing, kiln drying of Douglas fir is much quicker and allows control of the drying process so that even minor defects can be prevented. Experimental material used in this investigation was kiln dried.

A literature survey revealed that there have been few investigations into the drying behaviour of timber after water storage. The information contained in existing reports is contradictory, but the consensus is that water-storage is detrimental to both air and kiln drying.

Boutelje (1977) and Boutelje *et al*. (1978) reported on the drying properties of pine (*Pinus sylvestris* L.) and spruce (*Picea abies* L.) in Sweden following ponding and sprinkling for different time periods. The pine logs dried slowly when they had been ponded for longer than 14 weeks. Indeed, their pine logs were not dry enough for preservative

treatment even after two years air drying. With a ponding period of less than 14 weeks, the drying rate was not markedly different from that shown by unponded pine logs. The length of water-storage time required to affect the drying rate in pine was related to the season in which water storage was initiated i.e. shorter periods were required in summer than in winter. Spruce logs, on the other hand, could be water-stored for any length of time without affecting their rate of drying. Boutelje and Ihlstedt (1978) found no difference in the equilibrium moisture contents of water-stored and non-stored pine and spruce logs when they were dried under the same conditions.

Dalgas and Moltesen (1975) studied kiln drying characteristics of European Beech. They observed that initial drying to fibre saturation point was slower in their controls than in both sprinkled and ponded material. They also noted that the equilibrium moisture content (e.m.c.) of the controls was lower than that of water-stored logs. Differences were statistically significant but were thought to be of no practical importance because the timber under investigation was only 25mm thick.

In the early 1970's the Western Forest Products Laboratory in Canada examined the drying characteristics of softwood timber salvaged after 20 years submersion in several valleys flooded for hydro-electric power schemes. Moisture content gradients within flooded timber were very different from those of freshly felled timber (Salamon 1973). The uneven moisture distribution was thought to be responsible for the extensive ringshake and casehardening which occurred during conventional kiln drying schedules.

Concern was expressed at the prevalence of such defects and the potential for the subsequent down grading of water-stored timber on the market place (Salamon 1973, Dobie and Salamon 1973). Salamon (1973) found that the moisture content of flooded timber ranged from 100-215%, significantly higher than that of freshly felled material. It was thought that removal of the extra moisture would increase kiln drying costs (Smith 1975). Logs, broken down into 2" X 4" X 8' rough-sawn lumber, were air-dried for 1-2 years to reduce the moisture content to an acceptable level for further processing; however, during that time the timber had reached only 77-120% moisture content, whereas freshly felled timber dried under similar conditions reached a moisture content of 50-60% (Salamon 1973). Dobie and Salamon (1973) went so far as to say that the time required for air-drying of lake-stored wood made it economically non-viable.

Haslett (1980, unpublished) investigated the effect of two years' sprinkler-storage on the drying behaviour of 100 X 50mm radiata pine sawn timber. Timber was kiln dried under a schedule of 71°C dry bulb and 60°C wet bulb. Drying time, shrinkage and checking tendency were compared in sprinkled and non-sprinkled timber. The percentage moisture saturation was found to be significantly higher in sprinkled wood. No differences in the mean drying times from the green state to 15% m.c were observed. Tangential and radial shrinkage from the green state to 12% m.c. was also found to be similar between stored and non-stored material. However, when the samples were redried after preservative treatment, tangential shrinkage in the stored wood was found to be 16%

less than that in non-stored wood. Surface checking was more severe in stored wood after preservative treatment.

Lay Yee (1981) found that radiata pine posts which had been stored under water sprinklers for 27 months, air-dried faster (from green to 40% m.c.) than non-stored posts of similar dimensions. Lay Yee recommended that posts stored under sprinklers be air-dried under cover to avoid excessive absorption of rain water.

In summary, water-sprinkling and storage of logs in lakes or ponds can affect the drying properties of timber. Both the drying rate and the incidence of drying defects may be altered. It seemed pertinent to monitor those properties in the water-sprinkled Douglas fir used in this study. The remainder of this chapter is therefore devoted to the methodology and results relating to the measurement of drying rates, moisture content distribution during drying and the percentage shrinkage occurring after drying in sprinkled and non-sprinkled Douglas fir.

5.2 THE INCIDENCE OF CHECKING AND SHRINKAGE

Above the fibre saturation point, no significant dimensional changes occur in wood during drying. Below fibre saturation point, however, anisotropic shrinkage occurs. Shrinkage in a longitudinal direction is generally found to be negligible, hence volumetric shrinkage is almost entirely due to its radial and tangential components. Tangential shrinkage is usually 1.5 - 2 times greater than radial shrinkage. Several hypotheses have been proposed to explain anisotropic shrinkage. Longitudinal shrinkage is usually

explained on the basis of cellulose microfibril angles in the S2 layer of longitudinally orientated cell walls (Barber and Meylan 1964, Cave 1972). Explanations of transverse shrinkage have been reviewed by a number of authors including Kollman and Cote (1968, p.208) and Skaar (1972, p.99). Each theory attempts to explain shrinkage anisotropy on the basis of the physical attributes of wood such as the orientation of rays, the location of cell to cell pitting and the microfibril orientation in cell walls. In practice, all of the proposed mechanisms of shrinkage contribute in varying degrees to the overall behaviour (Panshin and de Zeeuw 1980, p.210).

Stresses in timber arising from hydrostatic tension and differential shrinkage are the main causes of drying defects. Differential shrinkage can arise from anisotropic shrinkage, steep moisture gradients or a combination of both.

The incidence of surface checks and splitting was examined in kerfed logs after conditioning to 10% e.m.c. Surface checking was minimal in both sprinkled and non-sprinkled logs and no attempt was made to quantify the observation. The kerf undoubtedly contributed to the very mild checking observed but the gentle drying schedule used was also an important factor.

A kerf in a log will normally open up as the wood dries below fibre saturation point (f.s.p.) because of shrinkage. Shrinkage of wood, and kerf widening , increase in proportion to losses in moisture content. Surprisingly, it became apparent that the kerfs in sprinkled logs did not open so wide as those in non-sprinkled logs, suggesting that

tangential shrinkage in sprinkled bolts is in some way reduced. To quantify the observation, the ratio of the kerf width to the kerf depth (log diameter/2) for each bolt was measured. Raw data are presented in Appendix R15 and summarised in Table 5-1. The data show that the kerf opened

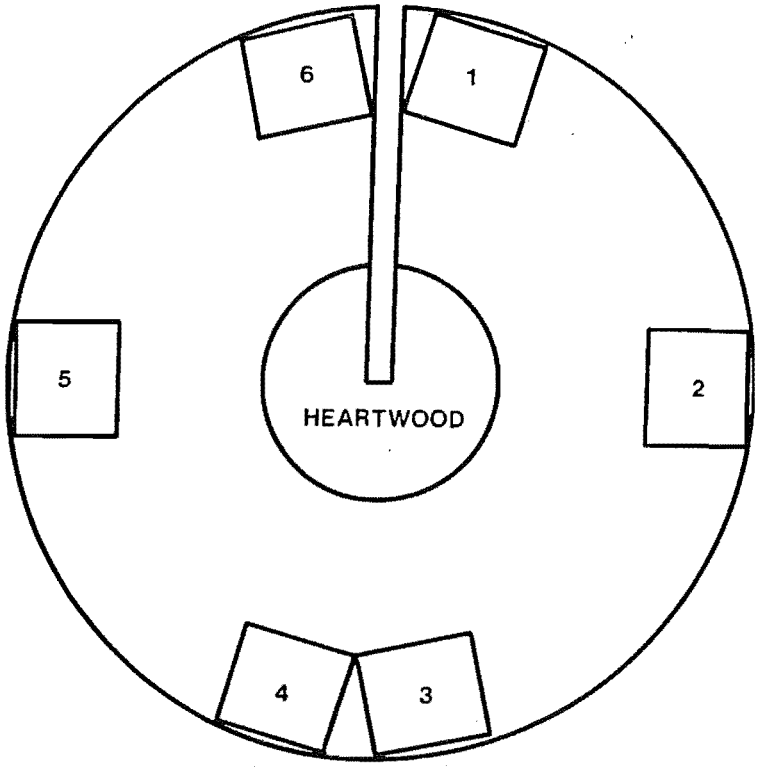
TABLE 5-1 KERF WIDTH/KERF DEPTH RATIOS IN SPRINKLED DOUGLAS FIR DRIED TO 10% E.M.C.

SPRINKLING TIME AND TREATMENT		KERF WIDTH/ KERF DEPTH	S.E.	LSD P=0.05
NON-SPRINKLED		0.264	0.008	N/A
WEEK 1	T 1	0.171	0.005	0.012
	T 2	0.174	0.002	
	T 3	0.179	0.005	
	T 4	0.208	0.007	
WEEK 2	T 1	0.207	0.011	0.021
	T 2	0.184	0.002	
	T 3	0.206	0.006	
	T 4	0.204	0.005	
WEEK 3	T 1	0.179	0.001	0.014
	T 2	0.184	0.002	
	T 3	0.189	0.004	
	T 4	0.175	0.001	
WEEK 4	T 1	0.197	0.005	0.022
	T 2	0.188	0.011	
	T 3	0.182	0.005	
	T 4	0.172	0.001	
WEEK 5	T 1	0.171	0.006	0.011
	T 2	0.170	0.001	
	T 3	0.191	0.002	
	T 4	0.214	0.005	
WEEK 7	T 1	0.184	0.003	0.014
	T 2	0.194	0.002	
	T 3	0.199	0.002	
	T 4	0.197	0.007	

less in sprinkled bolts. The least significant difference (LSD) values for each sprinkling time in Table 5-1 indicate that the width of the kerf opening was the same for each sprinkling treatment. More surprisingly, there is no obvious effect of sprinkling time on the kerf width to kerf depth ratio. The ratio has the same value at one week's and seven weeks' sprinkling, which was unexpected. The phenomenon is not due to differences in the equilibrium moisture content as the results in Appendix R15 indicate. To investigate the matter further an experiment was devised to compare radial and tangential shrinkage in cubes cut from sprinkled and non-sprinkled bolts.

Timber for the shrinkage measurements was prepared by sprinkling kerfed bolts for five weeks using the techniques already described (Chapter four, Section 4.2). A 1.8m pole was divided into six equal sized bolts. Four of those bolts were sprinkled and the remaining two were returned to cold storage. A nutrient solution containing nitrogen but no phosphate buffer was used (i.e. equivalent to treatment 2, Section 4.2). At the completion of sprinkling, discs 25mm wide were cut from the centre of each bolt. Two similar discs were cut from the non-sprinkled bolts. Six 25mm cubes were cut from each sprinkled disc and four cubes were cut from each non-sprinkled disc (Figure 5-1). Care was taken to ensure that all cubes were orientated so that the cut sides represented true axial, radial and tangential faces. The wet weights and dimensions of each cube were measured and recorded. The cubes were then laid out on a tray and allowed to dry slowly in a room where temperature and humidity were controlled at 24°C dry bulb and 20°C wet bulb giving 75%

Figure 5-1 Diagram showing the location of cubes removed for shrinkage measurements.



NOTE: Cubes 1-6 were removed from sprinkled bolts, cubes 1, 3, 4 and 6 from non-sprinkled bolts.

relative humidity. The weight of each cube was measured daily until it remained constant for three days; it was then assumed to have reached equilibrium moisture content (e.m.c.). The axial, radial and tangential dimensions were measured at e.m.c. and converted to percentage shrinkage using equation 5-1.

$$\text{Shrinkage \%} = \frac{\text{change in dimension from swollen size}}{\text{swollen dimension}} \times 100 \quad (5\text{-}1)$$

All cubes were then oven dried and the dimensions were remeasured at 0% moisture content. Raw data are presented in Appendix R16 and summarised in Table 5-2. Non-sprinkled

TABLE 5-2 MEAN RADIAL AND TANGENTIAL SHRINKAGE OF 5 WEEK SPRINKLED DOUGLAS FIR CUBES AT E.M.C. AND OVEN DRY CONDITION

	E.M.C.	RADIAL SHRINKAGE AT E.M.C.	TANGENTIAL SHRINKAGE AT E.M.C.	RADIAL SHRINKAGE AT 0% M.C.	TANGENTIAL SHRINKAGE AT 0% M.C
SPRINKLED	14.89 *	1.78 *	3.06 **	4.34 N.S.	6.83 **
NON-SPRINKLED	14.53	2.28	4.43	4.66	8.28

N.S. NO SIGNIFICANT DIFFERENCE BETWEEN SPRINKLED AND NON-SPRINKLED CUBES
* SIGNIFICANTLY DIFFERENT AT P=0.05
** SIGNIFICANTLY DIFFERENT AT P=0.01
*** SIGNIFICANTLY DIFFERENT AT P=0.001

cubes, C1-C8 can be considered replicate samples and for the purposes of statistical analysis the data for all of those cubes were grouped together. Initially it was considered that shrinkage of the cubes might be affected by the proximity of the kerf: cubes cut from comparable positions in the four replicate sprinkled bolts were to be analysed separately. However, statistical analysis showed that such a segregation was not necessary because position had no effect on shrinkage. Therefore the shrinkage measurements for the sprinkled cubes in Table 5-2) represent the means of 24 cubes.

Table 5-2 shows that the equilibrium moisture contents between sprinkled and non-sprinkled cubes are statistically different; however, for practical purposes that difference is negligible and it is still valid to compare shrinkage values. Axial shrinkage in both sprinkled and non-sprinkled

wood was negligible and the data set was omitted. The percentage radial and tangential shrinkage in sprinkled cubes at 14 % e.m.c is lower than that in non-sprinkled cubes. In oven dry wood, however, no difference in the radial shrinkage is evident between sprinkled and non-sprinkled cubes, but tangential shrinkage is 17% less.

The shrinkage experiment was repeated using cubes cut from bolts sprinkled for 12 weeks. Raw data are presented in Appendix R17 and summarised in Table 5-3. The results show

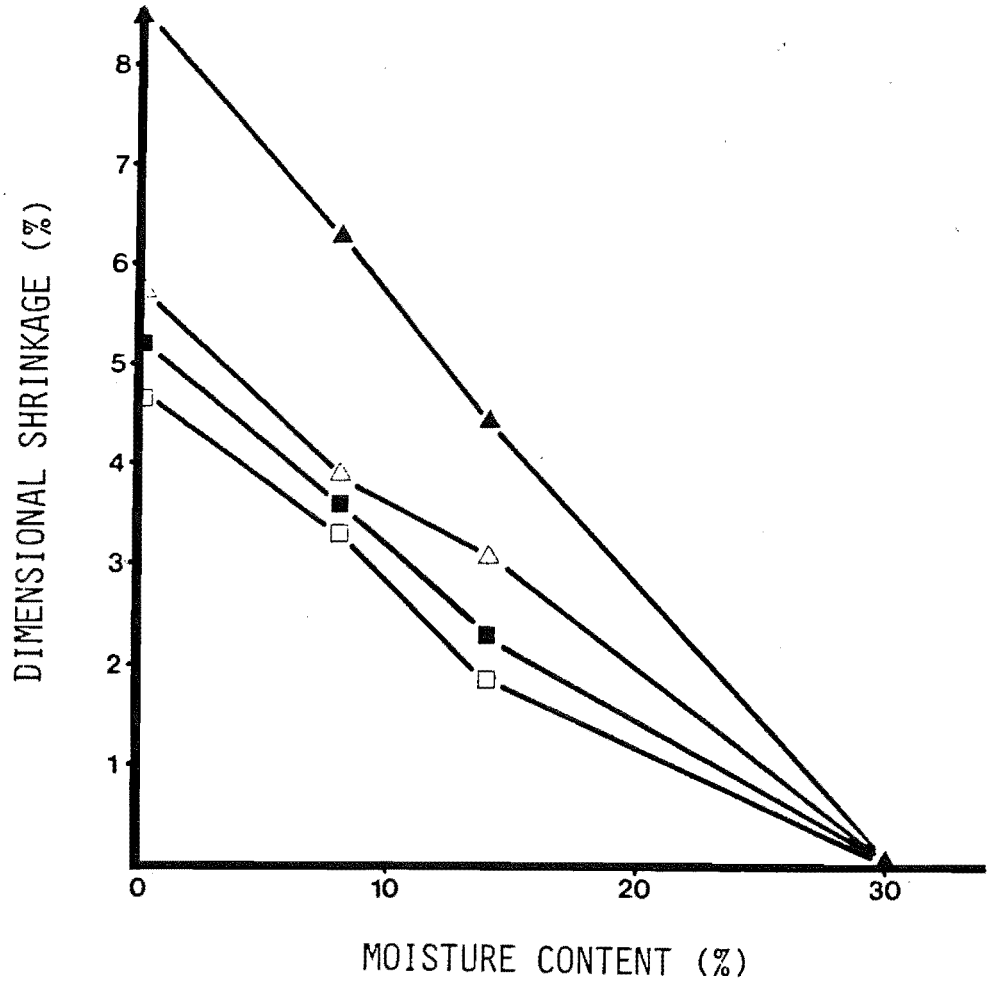
TABLE 5-3 MEAN RADIAL AND TANGENTIAL SHRINKAGE OF 12 WEEK SPRINKLED DOUGLAS FIR CUBES AT E.M.C AND OVEN DRY CONDITION

	E.M.C.	RADIAL SHRINKAGE AT E.M.C.	TANGENTIAL SHRINKAGE AT E.M.C.	RADIAL SHRINKAGE AT 0% M.C.	TANGENTIAL SHRINKAGE AT 0% M.C.
SPRINKLED	7.92 N.S.	3.3 N.S.	3.9 *	4.7 N.S.	5.7 ***
NON-SPRINKLED	7.92	3.6	6.3	5.2	8.5

N.S. NO SIGNIFICANT DIFFERENCES BETWEEN SPRINKLED AND NON-SPRINKLED CUBES
* SIGNIFICANTLY DIFFERENT AT P=0.05
** SIGNIFICANTLY DIFFERENT AT P=0.01
*** SIGNIFICANTLY DIFFERENT AT P=0.001

that there is no difference in the e.m.c. between sprinkled and non-sprinkled cubes. Note that in this instance the equilibrium moisture content was about 8%, 6% lower than the previous experiment, because of a difference in the drying climate. Sprinkling had no effect on radial shrinkage at either e.m.c. or oven dry condition. In contrast, tangential shrinkage was significantly reduced at both moisture contents. Figure 5-2 summarises the shrinkage results of

Figure 5-2 Radial and tangential shrinkage of sprinkled and non-sprinkled D. fir as a function of moisture content.



KEY TO SYMBOLS:

▲ Tangential non-sprinkled
△ Tangential sprinkled
■ Radial non-sprinkled
□ Radial sprinkled

both five and twelve week sprinkling experiments with the assumption that sprinkling for longer than five weeks results in no further significant change in shrinkage. The decrease in tangential shrinkage after sprinkling is obvious; so is the similarity between the radial shrinkage

curves for sprinkled and non-sprinkled wood.

Tangential and radial shrinkage are often quoted as a ratio (Panshin and de Zeeuw 1980, p.208). Ratios approaching unity imply very little opening of the kerf or very little checking in non-kerfed roundwood. Tangential to radial shrinkage ratios calculated for 12 week sprinkled wood from the green state to the oven dry state show a substantial drop i.e. from 1.6 to 1.2. Normal values quoted for coastal Douglas fir are generally in the order of 1.7 (Panshin and de Zeeuw 1980 p.499) which corresponds closely to the ratio for non-sprinkled cubes in this experiment.

The possibility that the reduction in tangential shrinkage was due to 'bulking' of the cell wall with absorbed nutrient salts was examined. It has already been shown that wood sprinkled with a nutrient solution absorbs noticeable quantities of salts (Section 4.3.2). Stamm (1964, p.257) outlines the effects of different concentrations of salts on wood shrinkage. The concentration of dry salt added to the original sprinkling medium was small, although once inside the wood, the salt solution became more concentrated as water was removed from cell lumina and cell walls during the drying process. Even so, it is difficult to see how 'bulking' could occur, because the quantity of wood present in each sprinkling system vastly exceeds the quantity of salt available for absorbtion. The following calculation provides a mathematical basis for discounting any effects of 'bulking':

The total wet weight of the wood present in the sprinkling tank was:

$$5 \text{ X } 8\text{kg} = 40\text{kg}$$

The moisture content of the wood at saturation was approximately 160%; therefore, 25kg of the 40kg wet weight is water and only 15kg is wood. The sprinkling tank contained 20 litres of solution to which 90g of $(NH_4)_2 SO_4$ was added. That quantity of salt (divided by the density of ammonium sulphate (1.77g/ml) and ignoring any water of hydration) converts to a volume of approximately 50ml. At fibre saturation point (when shrinkage starts to occur) only 4500ml of water is present in the cell walls of all the wood present in the sprinkling tank.

e.g. the amount of water present at fibre saturation (30% m.c.)

$$= \text{(wet weight - dry weight)} = \frac{30 \text{ X oven dry weight}}{100}$$

$$= 0.3 \text{ X } 15000$$

$$= 4500\text{ml}$$

Therefore only 50ml of salt is available to displace 4500ml of water present in the wood at f.s.p. Even if all that salt is absorbed into the wood, the maximum reduction in overall shrinkage due to 'bulking' at 0% moisture content cannot be more than:-

$$\frac{50}{4500} \text{ X } \frac{100}{1} = 1\%$$

Such a small effect can be ignored. Supporting evidence comes from the fact that bolts sprinkled without

added nutrients (i.e. treatment 4) show a reduction in shrinkage (Table 5-1).

5.3 THE EFFECTS OF SPRINKLING ON THE DRYING OF DOUGLAS FIR

5.3.1 Pathways for moisture movement in softwoods

The pathways available for moisture movement during the drying of wood are essentially the same as those discussed previously in relation to preservative penetration (Section 2.3, Chapter 2). The drying capability of timber is closely related to the timber's permeability. Permeability and drying rate are both influenced by the anisotropy of wood. Axial permeability is generally found to be 1000-10000 times greater than that in either the radial or tangential direction (Panshin and de Zeeuw 1980, p.207). Movement of water in an axial direction is expedited by the orientation of the tracheids. In a cube of wood more water will be lost from the axial faces than from the other faces. However, for all practical purposes radial and tangential drying are more important than axial drying because both roundwood and sawn timber are generally much longer in an axial direction.

Comstock (1970) proposed two models which support a close relationship between tangential and axial permeability, controlled by the number, size and distribution of bordered pits. He found that radial permeability was controlled independently of axial and tangential permeability.

When the possibility of radial movement of free water

and water vapour is considered, pathways capable of facilitating such movement are difficult to identify. However, the data presented in Section 5.3.3 provide a prima facie case for some mass flow in the radial direction. Radial facing bordered pits are rare and are generally found only in the first few rows of cells on the earlywood-latewood boundary (Panshin and de Zeeuw 1980, p.137). Not all tracheids are orientated so that all bordered pits face in a tangential direction but a significant contribution to radial movement of free water from poorly orientated tracheids is unlikely. Ray tissues should allow radial movement of fluids, but in practice that is not always the case, because ray-to-tracheid and ray-to-ray pits lack any discernable openings to conduct free water and they are also frequently occluded with cell debris (Section 2.5). Certain species such as Douglas fir possess ray tracheids which are interconnected through bordered pits. Ray tracheids could conceiveably contribute to some radial movement of free fluid, but they are uncommon and are usually encrusted with phenolic substances, effectively rendering them impassable (Liese and Bauch 1967). One other possibility for radial movement of free water is via the interstitial spaces between adjacent ray parenchyma (Bolton et al. 1975, Back 1969). Such spaces are common in Douglas fir; their efficacy for water movement depends on the permeability of the so called 'blind pits' which connect the spaces with the ray tissues.

5.3.2 Preliminary assessment of drying after sprinkling

Quartered Douglas fir bolts from the first sprinkling experiment (Section 3.3, Chapter three) were end and side sealed with epoxy resin while still wet. Once sealed, they were kiln dried using a mild drying schedule (34°C drybulb, 29°C wetbulb giving 75% r.h.). A mild kiln schedule was selected to reduce checking. Severe checking would have affected eventual preservative uptake.

Exact duplication of kiln conditions from one run to the next is difficult. Therefore, non-sprinkled material was dried concurrently to ensure that both treated and non-treated wood were subjected to the same conditions. At daily intervals the kiln was opened briefly to weigh the bolts. The initial moisture content of the bolts was calculated from the wet weight and oven dry weight of biscuits removed from either end of the log prior to drying. Raw weight loss data were converted into transient moisture content data using equation 5-2.

$$MC(t) = WT(t)/WT(i) \times (MC(i) - 100) + 100 \quad (5\text{-}2)$$

Where MC(t) = moisture content at time (t)
WT(t) = weight at time (t)
WT(i) = initial weight at beginning of drying
MC(i) = initial moisture content at beginning of drying

The moisture content data were then converted to relative moisture contents (E) using equation 5-3 (Rosen 1982) and the 'E' values were plotted against drying time (Figure 5-3(a)).

Figure 5-3(a)

Radial drying of quartered bolts from experiment one sprinkled for eight weeks.

KEY FOR SYMBOLS:

·····	Treatment 1	Incised, not sprinkled
- - - -	Treatment 2	Incised, no nutrients
- - -	Treatment 3	Incised, nutrients, cyclic sprinkling
·——·-	Treatment 4	Incised, nutrients, constant sprinkling
———	Treatment 5	Not incised, nutrients
· · · ·	Treatment 6	Not incised, not sprinkled

Figure 5-3(b)

Radial drying of quartered bolts from experiment two sprinkled for eight weeks.

KEY FOR SYMBOLS:

·····	Treatment 1	Incised, not sprinkled
- - - -	Treatment 2	Incised, no nutrients
- - -	Treatment 3	Incised, nutrients, cyclic sprinkling
———	Treatment 4	Incised, nutrients, constant sprinkling
———	Treatment 5	Not incised, nutrients
· · · · ·	Treatment 6	Not incised, not sprinkled

Figure 5-3(a)

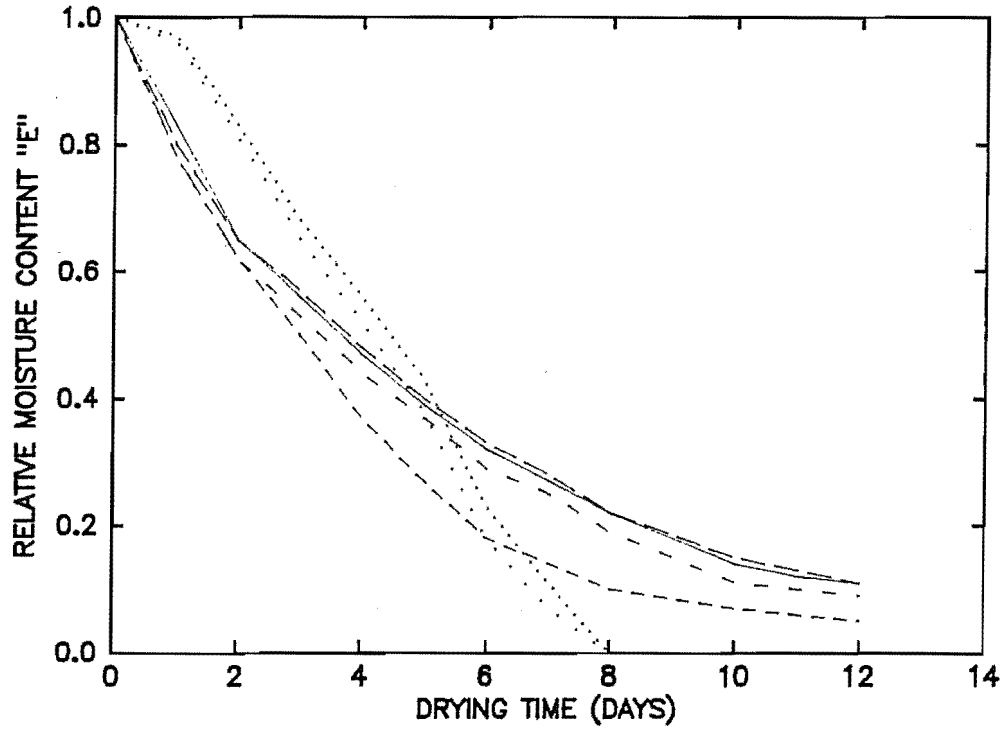
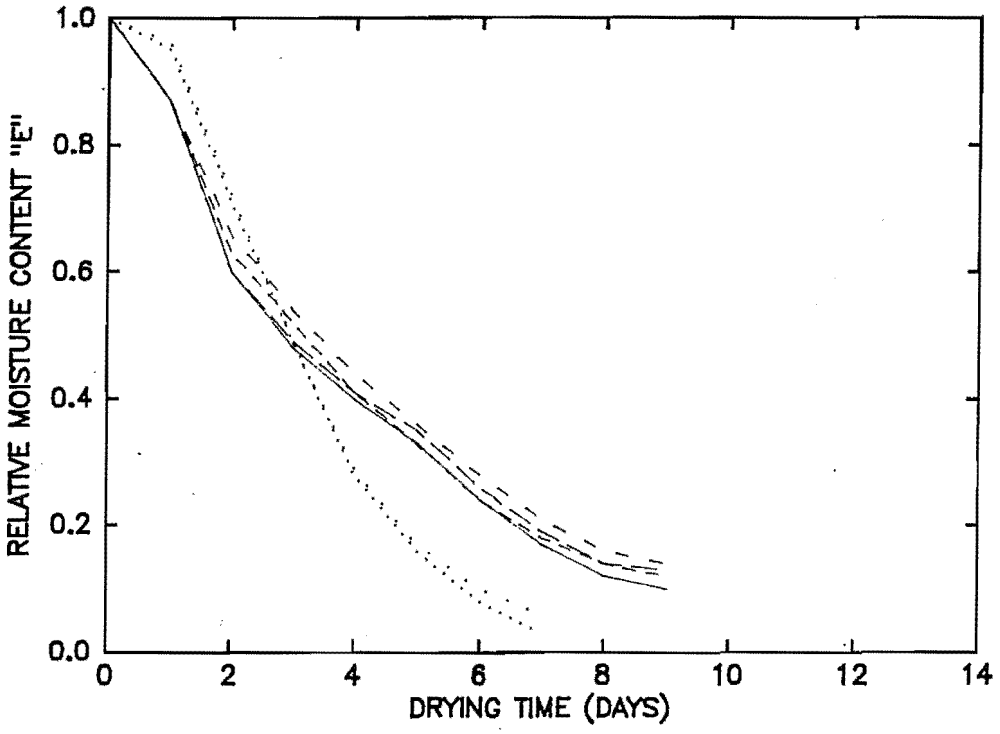


Figure 5-3(b)



$$E(t) = (MC(t) - E.M.C.) / (MC(i) - E.M.C.) \quad (5\text{-}3)$$

Where E(t) = relative moisture content at time (t)
E.M.C. = equilibrium moisture content at the drying conditions inside the kiln

The drying curves plotted in Figure 5-3(a) represent the rates of radial drying from a single exposed surface. Non-sprinkled controls dried faster than sprinkled material. There are no differences in the drying curves for sprinkling treatments 3, 4 and 5, but the curve for treatment 2 lies between that of the controls and the other treatments.

A light deposit of salt appeared on the exposed surfaces of the sprinkled bolts. It probably originated from the absorbtion of nutrients in the sprinkling solution and was left behind when moisture evaporated from the surface. Mild surface checking associated with the incisions occurred on all the incised bolts, both sprinkled and non-sprinkled.

Quartered bolts from experiment 2 (section 3.3, Chapter three) were also dried in a similar manner. The results are presented in Figure 5-3(b). Note that the only difference between experiment 1 and experiment 2 was the temperature at which the bolts were sprinkled. The curves in Figure 5-3(b) show that at the higher sprinkling temperature, the difference in drying time between controls and sprinkling treatments is not so great as it is in experiment 1. The curve for treatment 2 is no longer intermediate between the controls and the other sprinkling treatments. Unfortunately no direct comparison can be made between Figures 5-3(a) and 5-3(b) because the drying

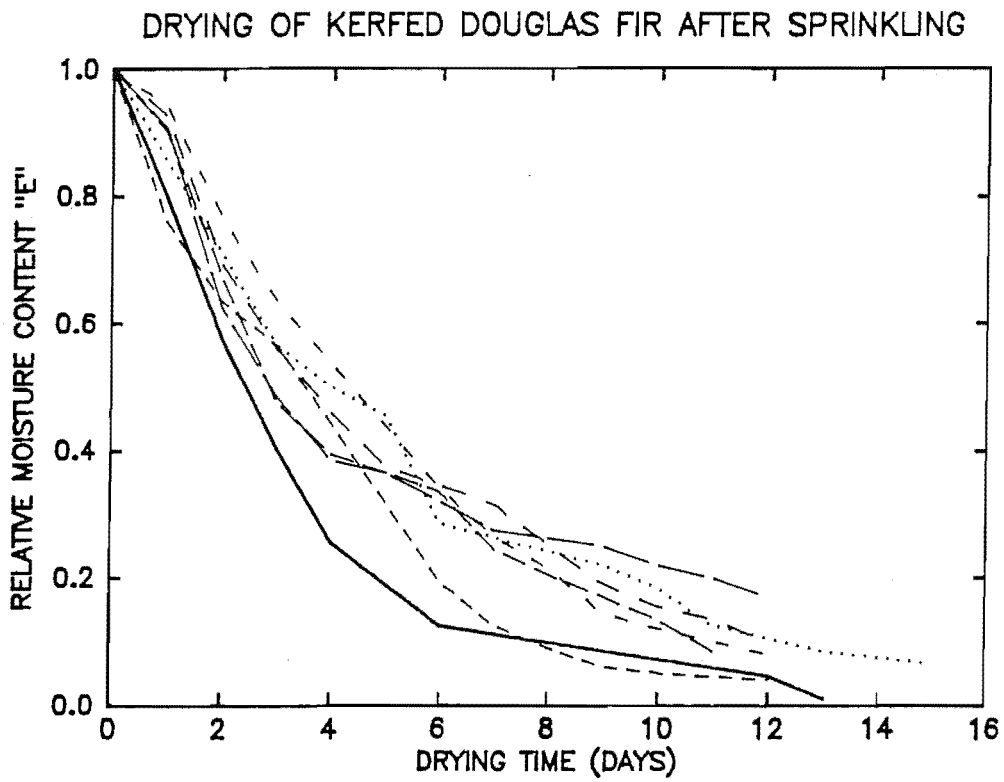
conditions inside the kiln differed slightly. Nevertheless it is still possible to show that increasing the sprinkling temperature resulted in an improvement in drying.

5.3.3 The effect of sprinkling time on drying time

The effect of sprinkling time on drying was examined using kerfed round wood (Section 4.3, Chapter four). Kerfed round wood was used for two reasons: firstly, drying quartered material was not considered to be comparable with the normal drying of round wood and secondly, kerfing would allow a certain amount of tangential drying. Kerfed wood was end-sealed in the same way as the quartered material.

Kerfed bolts were removed from the sprinkling tanks at weekly intervals and kiln dried using the same mild schedule already described (34°C d.b. and 29°C w.b. giving 75% r.h.). The drying curves obtained from the nitrogen and buffer treatment (treatment 3, Section 4.2) for each sprinkling time are expressed as relative moisture content 'E' and plotted against time in Figure 5-4. Drying curves for bolts from treatment 1, 2 and 4 are comparable but are not presented here. Figure 5-4 shows that sprinkled bolts dry more slowly than non-sprinkled bolts and that the reduction in drying rate is related to the sprinkling time. The curves suggest that from one week's to three weeks'sprinkling, the drying rate falls, reaching a minimum at three weeks. Thereafter, from four weeks onwards, the drying rate improves. However, at seven weeks' sprinkling,the drying rate is still less than that in non-sprinkled bolts. Interpretation of the result is

Figure 5-4



KEY TO SYMBOLS:

——— Control non-sprinkled

····· 1 week sprinkling

- - - - 2 weeks sprinkling

- - - 3 weeks sprinkling

— — — 4 weeks sprinkling

— — 5 weeks sprinkling

- —— - 7 weeks sprinkling

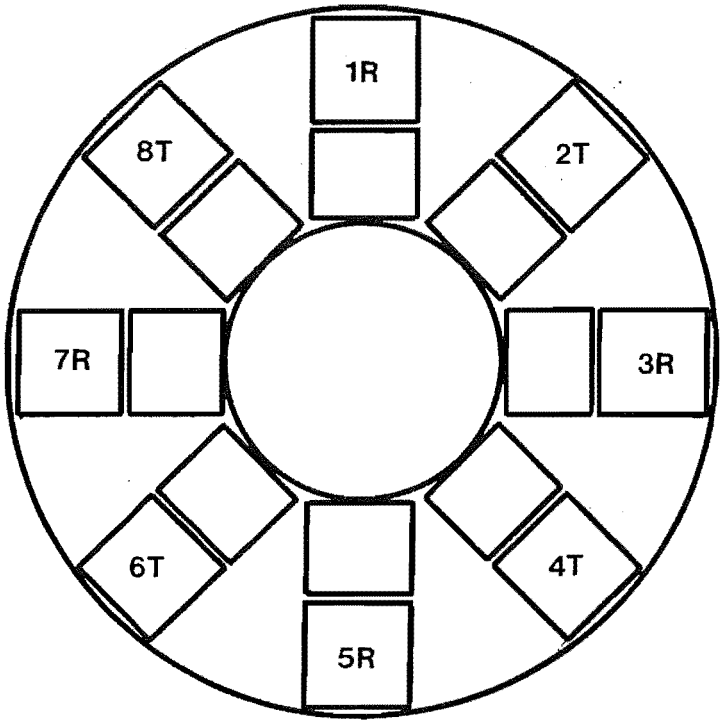
complicated by the fact that the curves in Figure 5-4 reflect both radial and tangential drying rates.

To determine exactly how sprinkling time and nutrient treatments affect drying, the unidirectional drying of small cubes from sprinkled wood was examined. Wood for the experiment was obtained from the sprinkling trial described in Section 4.3 (Chapter four). In that trial, round bolts were sprinkled over several weeks and with four nutrient treatments. Wood sprinkled for four, six and eight periods was selected for analysis. Non-sprinkled wood removed from cold storage was used as control material.

Discs were sawn from sprinkled wood and then machined into 22mm cubes as detailed in Figure 5-5. Care was taken to ensure that the grain of the timber was parallel to one side of the cube. Outer sapwood was differentiated from inner sapwood (Chapter four, Section 4.2). The wet weights of individual cubes were recorded before two pairs of opposing faces were sealed with petroleum jelly. Three of those faces were then covered with aluminium foil and the fourth was attached to a glass microscope slide. The microscope slides enabled the cubes to be handled without direct contact. Mounted cubes were dried in an air conditioned room at 23°C dry bulb and 19°C wet bulb giving approximately 65% relative humidity (Figure 5-6).

At approximately six hour intervals the weight of each cube was recorded. The true weight loss at each sampling time was calculated by subtracting the combined weight of the slide, petroleum jelly and aluminium foil (a constant) from the total weight. In that way it was possible to measure the rates of drying in axial, radial and

Figure 5-5 Diagram showing sampling points for drying cubes.



NOTE: R=radial T=tangential
Thus 1R,3R,5R,7R were used to measure radial drying rates and 2T,4T,6T,8T were used to measure tangential drying rates.

longitudinal directions. Raw weight loss data were converted into moisture content data using equation 5-2. The initial moisture contents of the cubes were originally assumed to be equal to the moisture contents of the adjacent wedges (Figure 5-5). However, those values were found to be too inaccurate and therefore initial moisture contents before drying had to be calculated after oven drying the cubes at the end of the experiment once e.m.c. had been reached.

Raw data for axial, radial and tangential drying of non-sprinkled outer sap cubes are presented in Appendix R18

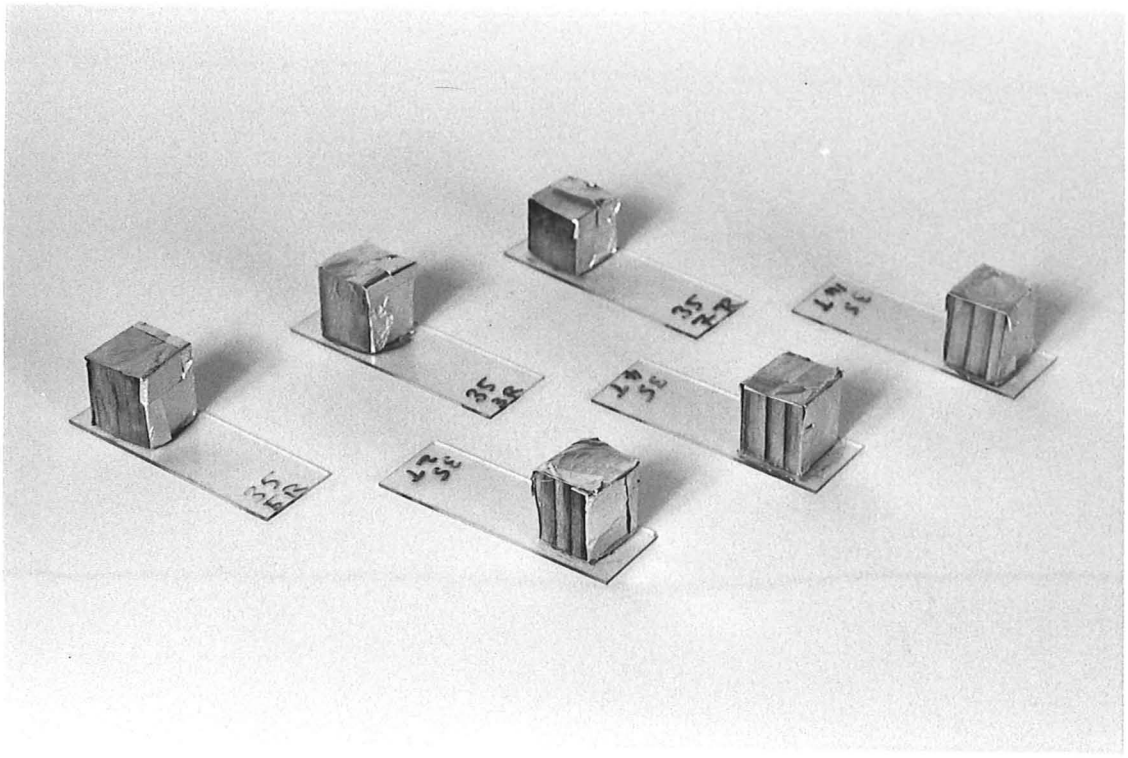


Figure 5-6 Photograph showing the method used to mount D. fir cubes for the experiment investigating radial and tangential drying rates.

and similar data for sprinkled outer sap material are provided in Appendix R19. Axial drying was not measured for sprinkled wood because axial moisture movement is of limited importance to the drying of whole logs. However, the data are included for non-sprinkled wood purely for comparative purposes. The data show that there is no difference in the equilibrium moisture contents of sprinkled and non-sprinkled cubes.

Tangential, axial and radial drying curves for non-sprinkled cubes are presented in Figure 5-7(a). The slope of the tangential drying curve is not so steep as the slopes of the radial and axial curves, suggesting that

Figure 5-7(a)

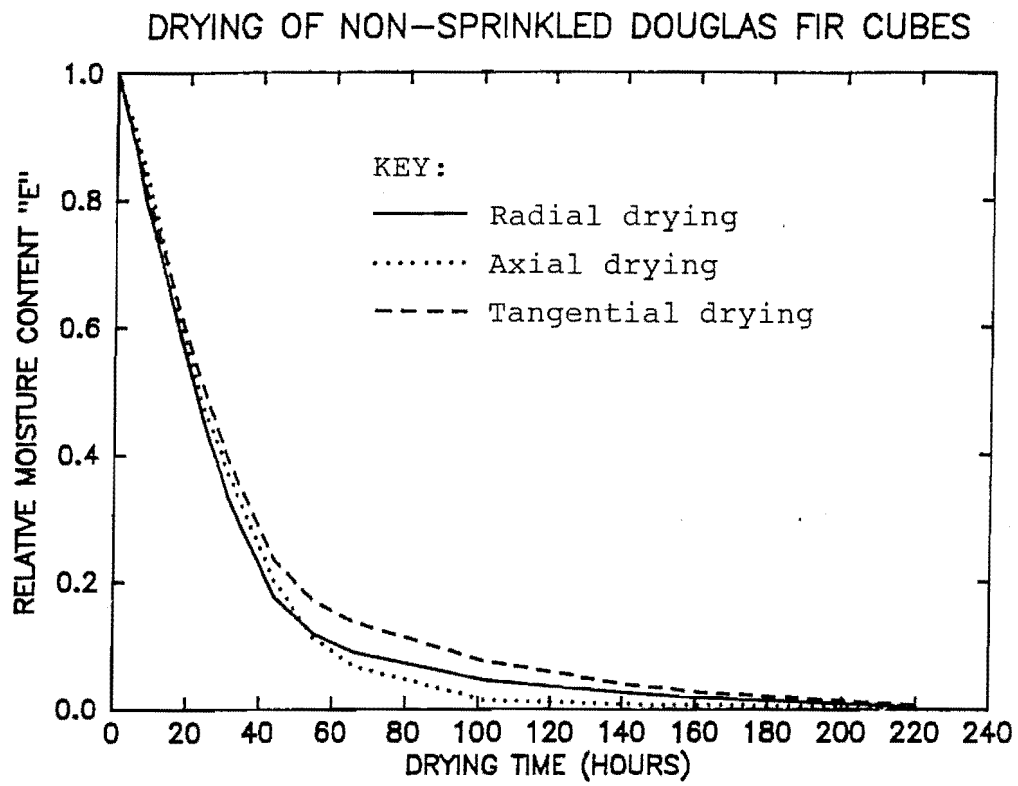
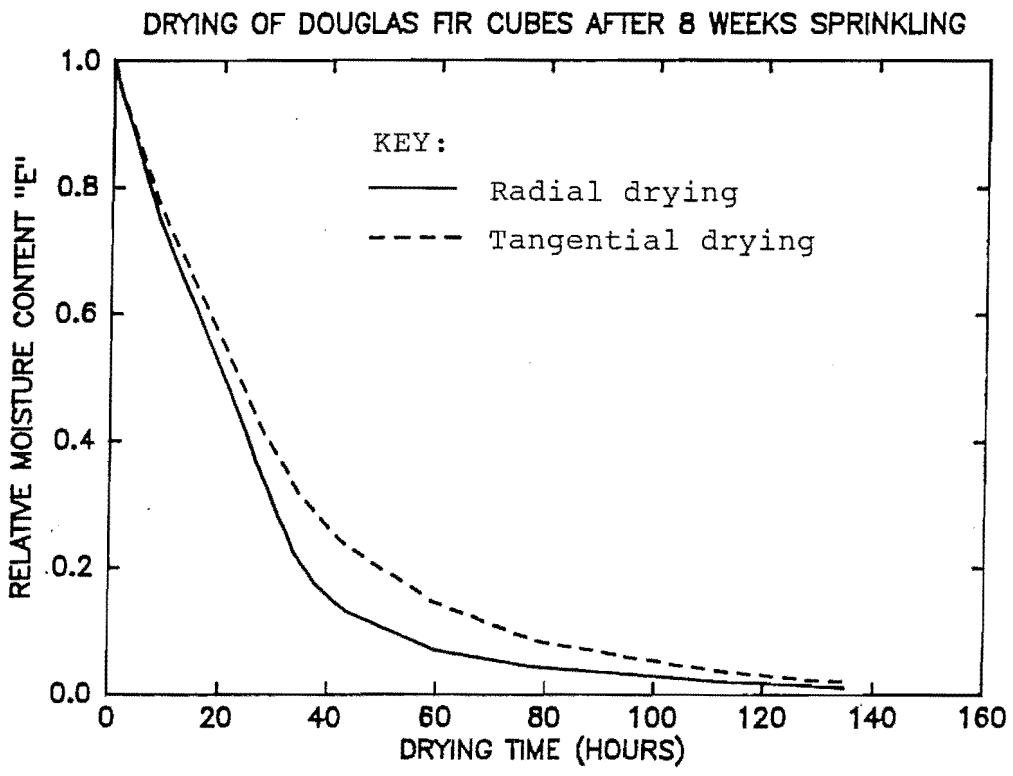


Figure 5-7(b)



tangential drying is slower. The curves for radial and tangential drying of cubes sprinkled with nitrogen and buffer (treatment 3) for eight weeks are presented in Figure 5-7(b) for comparison. Figure 5-7(b) shows that radial drying is again faster than tangential drying.

The similarity of the curves for radial and axial drying of non-sprinkled cubes is surprising, because the pathways for moisture movement in the two directions are thought to be very different. Theoretically, if mass flow of water can occur, then axial drying should be faster than radial drying because of the orientation of the tracheids in an axial direction. It is also surprising that radial drying is faster than tangential drying in both sprinkled and non-sprinkled cubes. The trends shown by the curves contradict established precedents and the figures merit further examination. Therefore, to compare the effects of sprinkling time and nutrient treatment on radial and tangential drying in Douglas fir, a model was constructed to describe the shape of the curves generated by the data in Appendices R18 and R19 (equation 5-4).

$$MC(t) = EXP(-(a + bt)) \qquad (5\text{-}4)$$

Where MC(t) is the moisture content at time (t) and (a) and (b) are coefficients characterising the shape and position of the curve.

Taking the natural logarithm of both sides of the equation gives the following linear relationship between moisture content and time (equation 5-5).

$$LN\ (MC(t)) = -(a + bt) \qquad (5\text{-}5)$$

Regression of LN(MC(t)) against drying time (t) converts the original drying curve into a straight line with slope (b) and intercept (a).

If moisture content data below fibre saturation point are included in the regression, the fit of the curve and the slope coefficient are affected considerably, because the moisture content versus time curve becomes assymptotic towards the equilibrium moisture content. Because the drying rate above fibre saturation is of interest, only moisture content data above that point were included in the regression. Regression lines fitted in this manner had R-squared values of 0.97 or greater. Thus, the slope coefficients are a reasonable measure of the drying rate above fibre saturation point.

Ideally the regression lines for each cube should have been compared by covariance analysis, but the complexity of the experimental design made that difficult. Instead the slope coefficients were compared by three-way analysis of variance (ANOVA) using the SPSSX statistical package; intercept coefficients were not considered important and were omitted. Analyses of variance were performed on radial and tangential drying data separately using sprinkling time, nitrogen and buffer concentration as independent variables.

Radial and tangential drying of non-sprinkled cubes was measured, but the data could not be included directly into the ANOVA. Non-sprinkling was not a valid treatment factor included in the experimental design, because there were no nutrient treatments associated with the

non-sprinkled cubes. One simple answer to the problem could have been to express the data as a ratio of the sprinkled drying rate over the non-sprinkled drying rate. Unfortunately those ratios are not in themselves normally distributed, a basic requirement of ANOVA. Normality could have been achieved through transformation, but transforming data twice is questionable. To resolve the dilemma, it was assumed that the drying rates for non-sprinkled cubes were constant and reflected normal drying rates for Douglas fir. By subtracting the control tangential constant from tangential sprinkled data and the control radial constant from radial sprinkled data, the drying rates could be expressed relative to 'normal' Douglas fir. Positive values were interpreted as an increase in drying rate and negative values as a decrease in drying rate. Rate differences were used as the dependent variable in the ANOVA.

Mean differences between sprinkled and non-sprinkled cubes for each sprinkling time and nutrient regime are tabulated in Table 5-4; summaries of the individual analyses of variance can be found in Table 5-5(a) and (b). For visual presentation of the data, the ratio of the sprinkled to non-sprinkled drying rate was considered more meaningful. Figure 5-8(a) and (b) show those ratios plotted against sprinkling time. It is evident that sprinkling time and nutrient treatment have similar effects on radial and tangential drying.

Unfortunately, the data set for week 3 is incomplete (radial and tangential drying rates for treatments 1 and 2 are missing). Nevertheless, Figure 5-8(a) and (b) show that

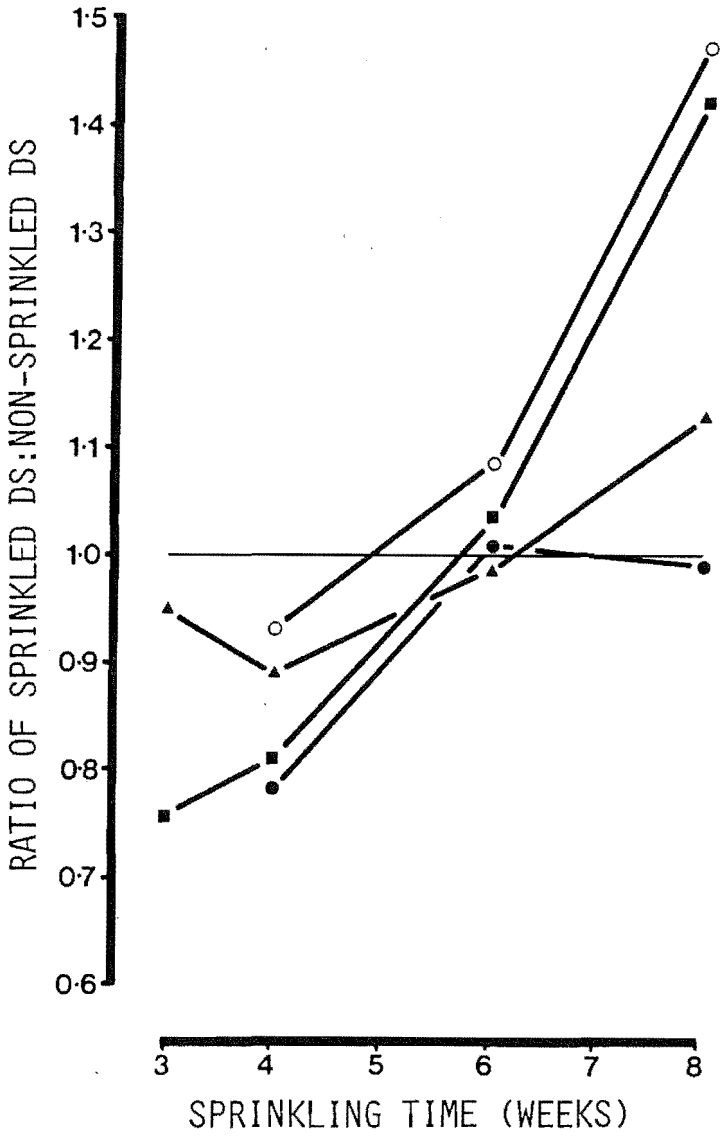
TABLE 5.4 MEAN DIFFERENCES IN RADIAL AND TANGENTIAL DRYING RATES BETWEEN SPRINKLED AND NON-SPRINKLED CUBES AFTER 4, 6 AND 8 WEEK SPRINKLING PERIODS AND FOR FOUR NUTRIENT TREATMENTS

SPRINKLING TIME (WEEKS)	NUTRIENT TREATMENT	DIFFERENCE IN RADIAL DRYING SLOPES	DIFFERENCE IN TANGENTIAL DRYING SLOPES
4	1	-0.0075975	-0.0049325
	2	-0.0022975	-0.0063700
	3	-0.0038975	-0.0063850
	4	-0.0061250	-0.0048475
6	1	-0.0008425	0.0029025
	2	0.0030825	0.0061325
	3	-0.0027800	0.0021075
	4	0.0009450	0.0044550
8	1	-0.0004400	0.0011275
	2	0.0168700	0.0119400
	3	0.0046225	0.0028250
	4	0.0150775	0.0116600

NOTE:-
NEGATIVE VALUES INDICATE DRYING RATES LOWER THAN NON-SPRINKLED MATERIAL
NUTRIENT TREATMENT 1 = BUFFER NO NITROGEN
NUTRIENT TREATMENT 2 = NITROGEN NO BUFFER
NUTRIENT TREATMENT 3 = BUFFER + NITROGEN
NUTRIENT TREATMENT 4 = NO BUFFER OR NITROGEN

the drying rates for treatments 3 and 4 after three weeks' sprinkling are lower than those in the non-sprinkled controls. Radial and tangential drying rates reach a minimum at 4 weeks. From four to six weeks' sprinkling, radial drying rates improved to a level equal to or greater than that shown by the non-sprinkled cubes. Between six and eight weeks, the drying rate for treatments 2 and 4 increased further. At eight weeks sprinkling the radial drying rates for treatments 2 and 4 were 47% and 43% respectively, greater than those for the non-sprinkled cubes. The radial drying rate for treatment 3 increased slightly, up to a level 13% higher than the controls, but

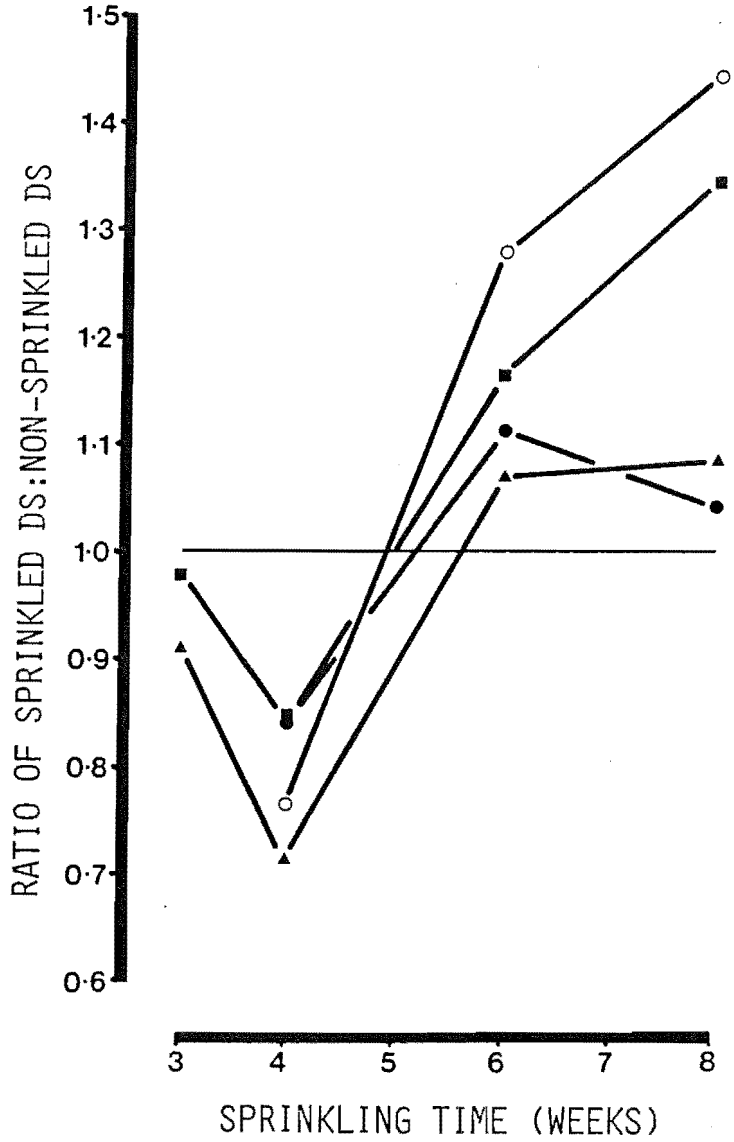
Figure 5-8(a) A comparison of radial drying slopes (DS) in sprinkled and non-sprinkled D. fir cubes at different sprinkling times.



KEY TO SYMBOLS:

●	Treatment 1	Buffer only
■	Treatment 2	Nitrogen only
▲	Treatment 3	Buffer and Nitrogen
O	Treatment 4	Water only

Figure 5-8(b) A comparison of tangential drying slopes (DS) in sprinkled and non-sprinkled D. fir cubes at different sprinkling times.



KEY TO SYMBOLS:

●	Treatment 1	Buffer only
■	Treatment 2	Nitrogen only
▲	Treatment 3	Buffer and Nitrogen
O	Treatment 4	Water only

the rate for treatment 1 fell. Similar increases can be calculated for the tangential drying rates.

The data in Figure 5-8(a) and (b) imply that treatments 1 and 3 reduce the effect of sprinkling on the drying rate. The result is interesting, because treatments 1 and 3 both contain phosphate buffer and treatments 2 and 4 do not.

The results of statistical analyses on the data shown in Figure 5-8 are presented in Table 5-5(a) and (b). Table 5-5(a) indicates that buffer, nitrogen and sprinkling time all affect the radial drying rate significantly. The interpretation of those effects is confounded by a significant interaction of buffer with sprinkling time. That is, the effect of buffer is not constant at all sprinkling times. There is no significant interaction between buffer and nitrogen and the three-way interactions are also insignificant. The ANOVA summary for tangential drying (Table 5-5(b)) reveals a similar trend except that nitrogen has no significant effect on drying rate.

To summarise, the effects of nutrient treatment and sprinkling time are similar on radial and tangential drying. In the early stages of sprinkling (up to four weeks), the drying rate of Douglas fir is retarded. The drying rate increases to a level significantly above 'normal wood' as the sprinkling time increases. Despite the significant interaction between buffer treatment and sprinkling time, it appears that the presence of buffer diminishes the effect of sprinkling on both radial and tangential drying rates.

TABLE 5.5(a) SUMMARY OF THREE WAY ANOVA COMPARING THE RADIAL DRYING RATE WITH SPRINKLING TIME, AND NUTRIENT TREATMENT

SOURCE OF VARIATION	SS	DF	MS	F	SIG.
SPRINKLING TIME (ST)	1610.201	2	805.100	139.601	***
BUFFER TREATMENT (B)	493.763	1	493.763	85.616	***
NITROGEN TREATMENT (N)	70.883	1	70.883	12.291	***
N X B	0.290	1	0.290	0.050	N.S.
ST X B	345.095	2	172.548	29.919	***
ST X N	32.811	2	16.406	2.845	N.S.
ST X B X N	27.025	2	13.512	2.343	N.S.
EXPLAINED	2582.939	11	234.813	40.716	***
ERROR	207.618	36	5.767		
TOTAL	2790.557	47	59.374		

TABLE 5.5(b) SUMMARY OF THREE WAY ANOVA COMPARING THE TANGENTIAL DRYING RATE WITH SPRINKLING TIME, AND NUTRIENT TREATMENT

SOURCE OF VARIATION	SS	DF	MS	F	SIG.
SPRINKLING TIME (ST)	1368.589	2	684.295	123.220	***
BUFFER TREATMENT (B)	213.785	1	213.785	38.496	***
NITROGEN TREATMENT (N)	0.004	1	0.004	0.001	N.S.
N X B	0.323	1	0.323	0.058	N.S.
ST X B	203.358	2	101.679	18.309	***
ST X N	13.536	2	6.768	1.219	N.S.
ST X B X N	7.804	2	3.902	0.703	N.S.
EXPLAINED	1807.399	11	6164.309	29.587	***
ERROR	199.923	36	5.553		
TOTAL	2007.323	47	42.709		

The causes of retardation and eventual improvement in the drying rate of sprinkled wood require further consideration. In the early stages of sprinkling, bacteria preferentially attack bordered pits between tracheids (Chapter four, Section 4.5). Degradation of bordered pit membranes should affect tangential drying, but should have minimal influence on radial drying. There is little microscopic evidence to suggest that tracheid-to-ray pits or ray parenchyma-to-parenchyma pits are damaged sufficiently to account for the effects on radial drying (Section 4.5). It is possible that the bacteria remove encrusting materials from the tracheid-to-ray pit membranes, possibly improving radial drying. However, because those materials occur mainly inside the ray cells, the pit membranes themselves would have to be breached. Destruction of tracheid-to-ray pit membranes was not common in the early stages of sprinkling (Section 4.5).

Accumulation of bacterial biomass or the products of bacterial metabolism seem to be the most likely explanation for the decrease in drying rate at four weeks sprinkling. An attempt was made to relate drying rates to the size of the bacterial population present in the wood at the commencement of drying. The numbers of bacteria present in wood at different sprinkling times have already been presented (Chapter 4, Section 4.4.4). A graph of bacterial numbers against drying rate (not shown here) showed only a weak relationship and no statistical analysis seemed warranted. Of course, the bacterial counts made in Section 4.4.4 were a measure of only the living bacterial population. Dead cells could also contribute significantly to physical obstruction

of pathways for moisture flow. It is more likely, however, that a combination of bacterial cells, both dead and alive, and mucilaginous polysaccharide gums are responsible for the decrease in drying rate. There did not appear to be a simple assay available to measure the quantity of polysaccharide gums present in sprinkled wood and therefore a causal relationship with the retarded drying rate could not be verified.

When the moisture content of softwood timber approaches fibre saturation point, the bordered pits aspirate, sealing off adjacent tracheids (Petty 1972). Pit aspiration is only partially reversible after rewetting and it results in a loss of permeability (Thomas and Nicholas 1966). Once the continuity of the capillary network is broken by pit aspiration, mass flow is prevented and drying occurs more slowly through diffusional processes. Theoretically, it should be possible to use that phenomenon to obtain a qualitative measure of the degree of pit destruction attributable to bacteria. If the bordered pits in sprinkled timber have been sufficiently damaged to prevent proper aspiration, then the change should be reflected in the drying curves.

To examine that possibility, non-sprinkled cubes, cubes sprinkled for three weeks and cubes sprinkled for four weeks (from the first drying experiment) were resaturated with water and then dried. Almost complete saturation was achieved by submerging the cubes in distilled water and applying alternate cycles of vacuum (-80kPa) and atmospheric pressure. Once saturated the cubes were redried under the same environmental conditions used earlier (23°C d.b. and

19°C w.b.). Drying rates for each cube were calculated as already described and compared with the corresponding data from the first drying experiment. Results were expressed as the ratio of the initial drying rate/resaturated drying rate and are presented in Table 5-6. Assuming that all the

TABLE 5-6 RATIO OF GREEN DRYING SLOPE/RESATURATED DRYING SLOPE FOR DOUGLAS FIR CUBES

SPRINKLING TIME	DRYING DIRECTION	MEAN DRYING SLOPE GREEN-DRY	RESATURATED-DRY	RATIO
NON-SPRINKLED	RADIAL	.03545	.01132	3.13
	TANGENTIAL	.02683	.00562	4.80
3 WEEKS TREATMENT 4	RADIAL	.02727	.01925	1.41
	TANGENTIAL	.02628	.01195	2.20
3 WEEKS TREATMENT 3	RADIAL	.03357	.02010	1.67
	TANGENTIAL	.02471	.01187	1.83
4 WEEKS TREATMENT 3	RADIAL	.03180	.00899	3.53
	TANGENTIAL	.01923	.00565	3.40

bordered pits in the non-sprinkled cubes were undamaged and were able to aspirate, the ratios for non-sprinkled cubes can be assumed to represent the 'worst' situation. Ratios for sprinkled cubes smaller than the ratios for the 'worst' case can be interpreted as evidence of pit damage.

Table 5-6 shows that both radial and tangential drying rates fall considerably in non-sprinkled cubes after resaturation, suggesting that bordered pits are involved in both radial and tangential drying. A similar effect can be seen if the 'green-dry' and 'resaturated-dry' drying slopes

are compared after three weeks' sprinkling. The ratios of the drying slopes after three weeks sprinkling are approximately half those of non-sprinkled cubes. Because the 'green-dry' drying slopes do not differ greatly from those of non-sprinkled cubes, the decrease in the ratios must be due to faster drying after resaturation. The data show that the effect is greatest on tangential drying, which can be interpreted to mean that some damage to bordered pits has occurred. After four weeks' sprinkling the effect is confounded by the fact that the 'green-dry' drying slopes are considerably lower than the same slopes for the non-sprinkled cubes.

The improvement in drying after resaturation is perhaps better illustrated graphically. Graphs showing the change in moisture content over time for cubes dried from green and resaturated states are presented in Figure 5-9(a) and (b). The reduction in the tangential drying rate after resaturating non-sprinkled cubes is considerable, but much less in three weeks' sprinkled cubes. Had time permitted, curves for eight weeks' sprinkled cubes would have provided interesting comparisons.

Evidence supporting the possibility of pit damage can also be gained from an examination of moisture gradients in wood during drying. Tangential moisture gradients in:

[1] non-sprinkled, dried and rewetted cubes
[2] sprinkled but never dried cubes and
[3] sprinkled, dried and rewetted cubes

were measured at well spaced time intervals. The resulting curves can be compared in Figure 5-10(a), (b), and (c). The

Figure 5-9(a)

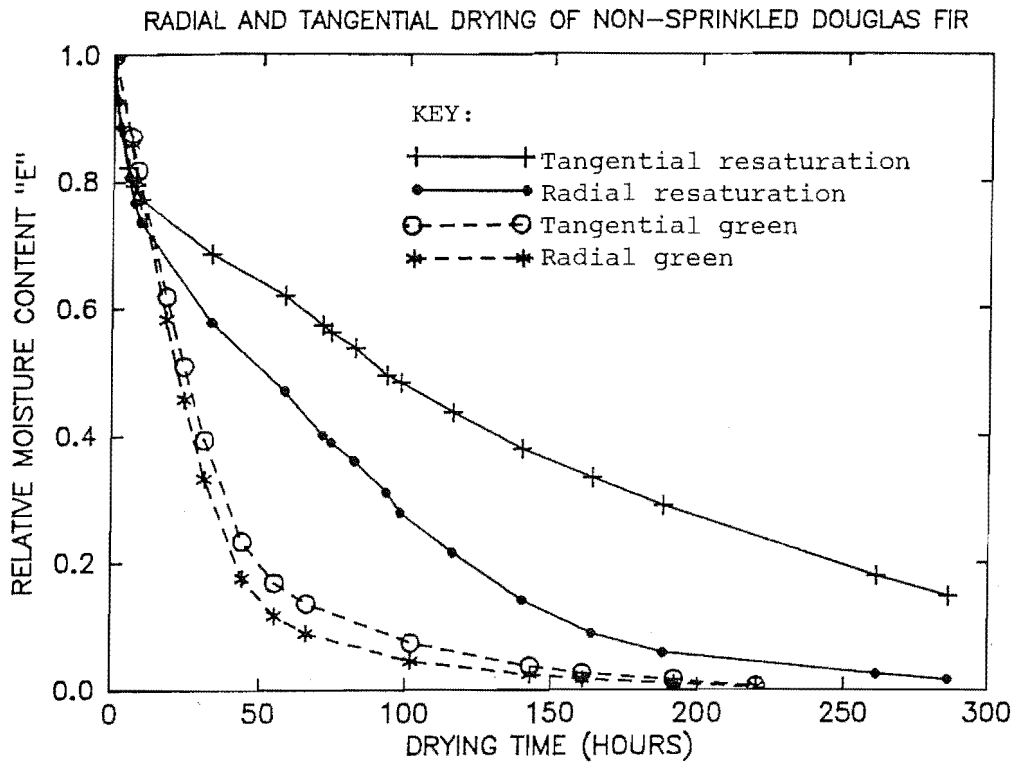


Figure 5-9(b)

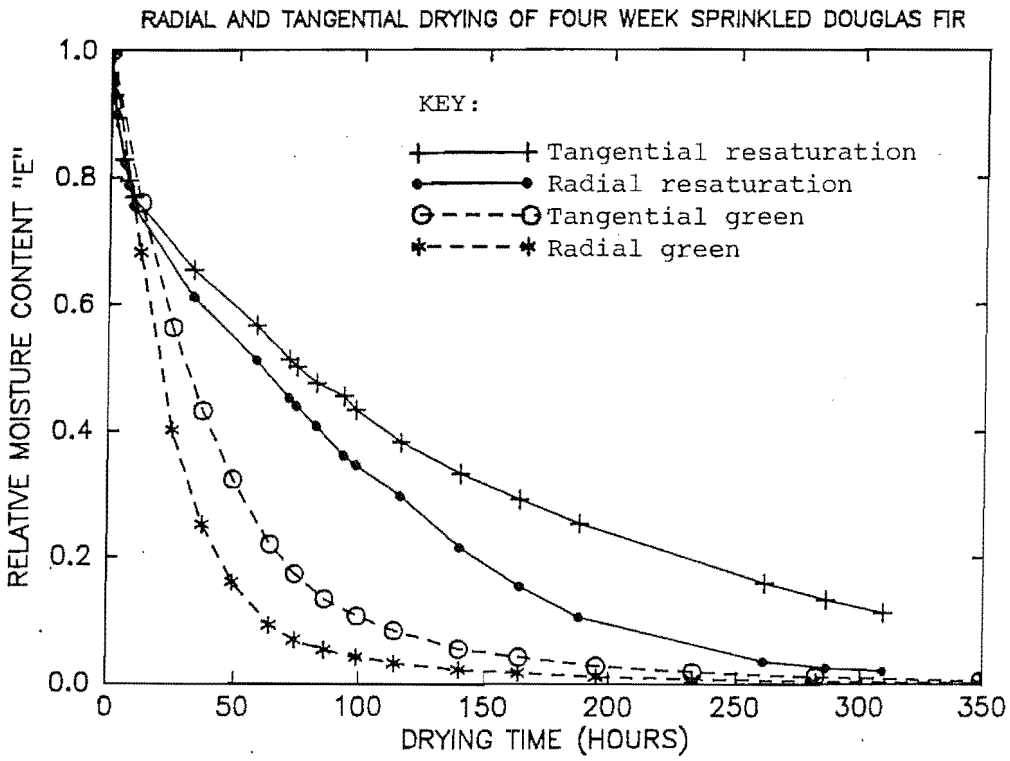


Figure 5-10

Tangential drying of Douglas fir cubes -
moisture gradients at different times.

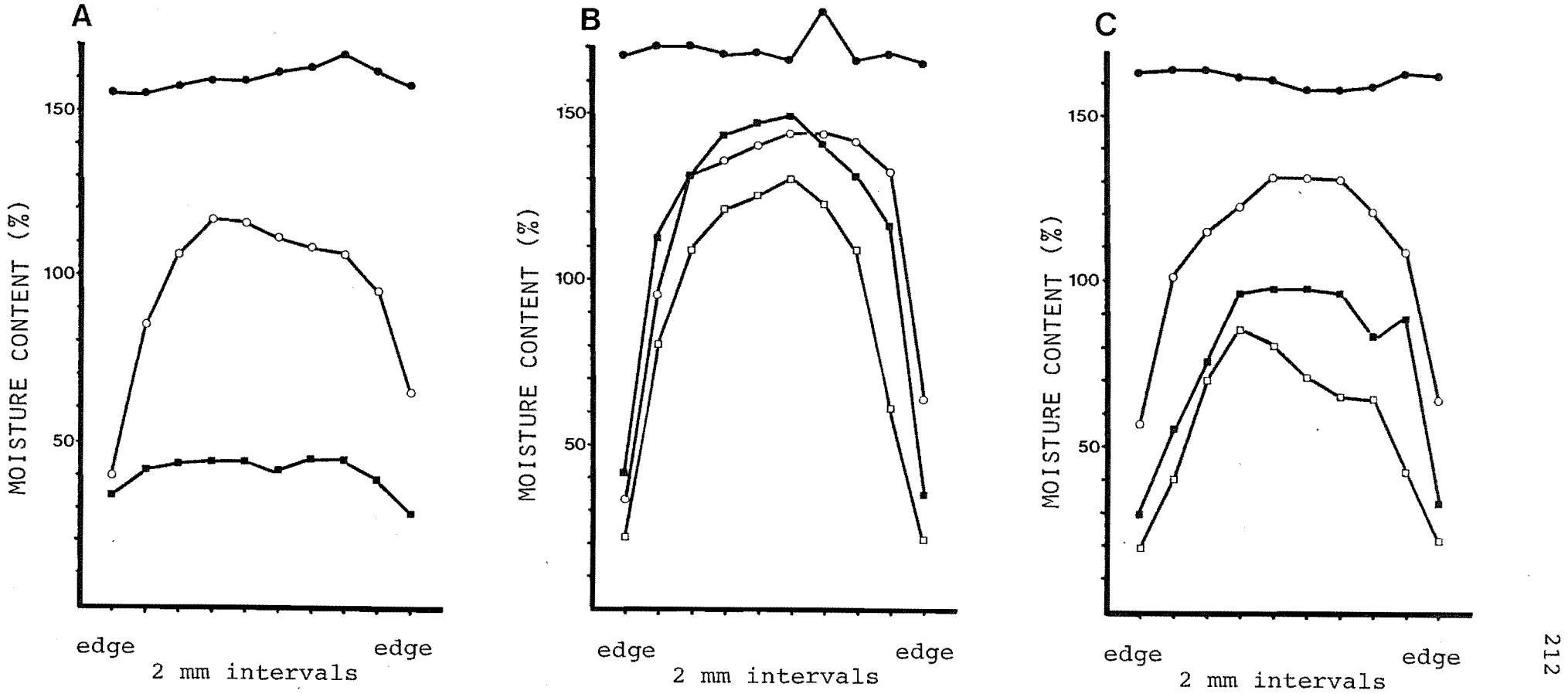
Graph (A) - sprinkled 'green' cubes

Graph (B) - non-sprinkled, dried and rewetted cubes

Graph (C) - sprinkled, dried and rewetted cubes

KEY TO SYMBOLS:

- ● Time = 0 hours
- ○ Time = 9 hours
- ■ Time = 21 hours
- □ Time = 48 hours


A
B
C
MOISTURE CONTENT (%)
150
100
50
edge
edge
2 mm intervals

moisture gradients for sprinkled but never dried wood (Figure 5-10(a)) correspond closely to an example given by Hart (1968) of a highly permeable wood drying by mass flow. Figure 5-10(b) shows the tangential moisture gradients in non-sprinkled resaturated wood and they are typical of impermeable woods in which no mass flow is occurring. Moisture gradients in timbers drying without mass flow are very steep, indicating that the surface layers reach e.m.c. while the centre is still saturated (Hart 1968). The poor drying can be explained by aspiration of bordered pits. Moisture gradients in sprinkled resaturated wood (Figure 5-10(c)) are intermediate between those in Figure 5-10(a) and (b) suggesting that at least some mass flow is occurring. That can be explained only if the bordered pits have been damaged.

If the moisture gradient under tangential drying exemplifies mass flow, then by inference mass flow must also occur in the radial direction.

CHAPTER 6

INTERPRETATION AND DISCUSSION OF THE RESULTS

This thesis originally proposed to investigate how water sprinkling of New Zealand grown Douglas fir can improve its permeability to water-borne preservatives. The project proved to be challenging for two reasons; first, because it was necessary to learn and use a wide range of different skills and second, because the results might be commercially applicable. The layout of this thesis does not follow a standard format but rather it reflects the evolution of ideas which led to the successful preservative treatment of Douglas fir sapwood with copper-chrome-arsenate. The purpose of the following discussion is to link those findings together.

Chapter one outlined the case for improving the treatability of Douglas fir. It concluded that water-storage under sprinklers was the most promising option to improve treatability. Considerable research has been undertaken to establish the mechanisms by which permeability is improved during water-storage, but the process is still not completely understood. There has been little commercial application of the process for two major reasons:

[1] the time taken to achieve adequate improvement in permeability is excessive and consequently uneconomic

[2] control of the process is difficult and therefore the results are variable

Chapter two discussed factors affecting permeability of softwood timbers in general and with specific reference to New Zealand grown Douglas fir. Douglas fir is considered to be a difficult-to-treat species but it is important to stress that this thesis is primarily concerned with the uptake of water-borne preservatives in sapwood. In North America, where Douglas fir is also considered refractory, heartwood permeability to creosote is of greater concern. Douglas fir sapwood is relatively easy to treat with creosote.

The refractory character of Douglas fir arises from its anatomical properties. The ray tissues are particularly impermeable which accounts for the poor radial preservative penetration and distribution in roundwood poles. Half-bordered pit membranes linking ray parenchyma to adjoining tracheids and the simple pits between ray parenchyma are usually encrusted and are therefore impassable. Ray tracheids, theoretically capable of allowing unimpeded radial movement of fluids, are uncommon and ray tracheid bordered pits linking adjacent cells are generally encrusted and blocked with debris (Liese and Bauch 1967). Axial and tangential movement of fluids in green Douglas fir is adequately facilitated by the bordered pits between adjacent tracheids. In all softwood species those same pits aspirate as the wood dries so decreasing the wood's permeability.

It has been suggested that the single most important factor affecting the permeability of wood is the size of the pores in the pit membranes. An equation relating the pressure required to force preservative fluids into dry wood

to the size of the pore radii was presented in Section 2.4. The equation predicts that small increases in the size of the membrane pores will have a very marked effect on permeability. It is well known that bacteria invading wood in water-storage attack the pit membranes, increasing pore sizes and as a result improving permeability.

Evidence of bacterial attack on pit membranes was presented in Section 4.5, Chapter four. Figures 4-27 to 4-30 suggested that the process of pit degradation followed a well defined chronological sequence. It was apparent that bordered pit membranes were degraded before half-bordered pit membranes. There was little evidence of damage to simple pit membranes. Similar observations have been made by other workers in water-stored wood. For example, Johnson (1979) noted that little damage occurred in the half-bordered pit membranes of Abies grandis and Douglas fir after 16 weeks exposure to Bacillus polymyxa. In contrast most of the bordered pit membranes were completely degraded. It is obvious that the length of time that pit membranes are exposed to bacterial attack governs the extent of their degradation. Unfortunately, exposure time was not the same in different areas of the wood. For example, at any particular moment in time, wood close to the surface of a sample had been exposed to bacterial attack for longer than wood at the heart-sap boundary. Examination of many samples of bacterially colonised wood revealed that most if not all the similar pits within a small SEM specimen (approximately 3-5mm cubes) were in a similar stage of decomposition. For example, all the bordered pits in several adjacent tracheids were either showing some signs of degradation or the

bordered pit had been completely removed (see Figure 4-29(a)). Often bordered pit membranes were completely missing while those adjacent to them were present but granular in appearance. It was assumed in such circumstances that the complete absence of a pit was a preparation artifact and that had the missing membranes been present they would have exhibited a similar degradation pattern to the others.

The degradation sequence can be related to the accessibility of the different membranes to bacterial enzymes. At a molecular level accessibility of pit membranes to enzymes is dependent not only on the ultrastructure and chemical composition of the membrane (discussed in Chapter two), but the molecular size and type of enzymes produced by the colonising bacteria. At a macroscopic level it is dependent on whether an enzyme can physically make contact with the membrane substrate.

In Chapter two it was stated that the principle chemical components of the bordered, half-bordered and simple pits were pectin, cellulose and to a lesser extent hemicellulose. Therefore the production of pectinases and cellulases by the bacteria will enable them to degrade pit membranes. Preliminary screening trials in Chapter three indicated that cellulases were not produced in-vitro by the mixed bacterial population used to inoculate the logs. Cellulolytic activity was not actually assayed in the sap squeezed from sprinkled bolts, but its absence can be inferred because the cellulose microfibrils of the bordered pit margo were not degraded (see Figures 4-27, 4-28 and 4-29, Chapter four). It has been reported in several studies

that cellulase activity by bacterial cultures isolated from water-stored wood or in sap squeezed from that wood is low or not detected (Karnop 1972(a), Ward and Fogarty 1973, Degroot and Sachs 1976). The absence of cellulase activity observed in this study is interesting because it has been reported that one of the organisms present in the mixed bacterial inoculum, Bacillus polymyxa, produces cellulase (Greaves 1971(b), Schmidt and Dietrichs 1976), although cellulase production by B. polymyxa is generally thought to be weak or lacking (Buchanan and Gibbons 1975).

In contrast to the lack of cellulase activity, considerable pectinase activity was detected in the sap squeezed from sprinkled bolts (Section 4.4.5, Chapter four). Evidence of pectinase activity can be seen in Figures 4-28 and 4-29 which show degradation of the pectin rich torus of the bordered pit membranes and also the central part of the tracheid-to-ray half-bordered pits. There are a number of so called 'pectic' enzymes which catalyse a variety of chemical reactions on so called 'pectins' (Fogarty and Ward 1972(a)). The nomenclature of the enzymes has been confused for some time but was recently reviewed by Lubomira and Markovic (1976). The pectic enzyme assayed in Section 4.4.5 performs a transeliminative cleavage of the D-(1-4) glycosidic bonds in D-galacturonan and consequently it can be termed a polygalacturonate transeliminase or a pectate lyase (Lubomira and Markovic 1976). The distinction is important because different pectic enzymes have different molecular weights. The molecular weight of an enzyme can determine its access to a substrate. The molecular weight of a pectate lyase isolated from Clostridium felsineum has been reported

as being 105,000, clearly a large molecule.

There has been much comment in the literature suggesting that the pit membranes in Douglas fir exhibit varying degrees of susceptibility to enzyme attack (Bauch et al. 1970, Meyer 1974). One explanation is that the bordered pits in Douglas fir have different chemical compositions. Bauch et al. (1970) suggested that differences in the susceptibility of Douglas fir bordered pit tori to enzyme attack could be related to the quantity of phenolic encrusting substances present in the membrane. A similar assertion was made by Bauch and Berndt (1973) after examining bordered pit tori using microspectrophotometric ultraviolet absorption techniques. Lignification of the pits does not occur until heartwood formation (Bauch and Berndt 1973, Saka et al. 1981). The apparent absence of lignin in sapwood pits is important because lignification of woody tissues prevents bacterial attack (Liese 1970, Schmidt and Liese 1982).

The presence of phenolic compounds in the bordered pit tori of Douglas fir is thought to retard or prevent enzyme attack. Much of the evidence in support of that argument comes from studies which have examined the susceptibility of the membranes to enzyme attack before and after mild extraction procedures to remove those phenolic compounds. In young pit membranes the phenolics are sparingly soluble and of low molecular weight (Nicholas and Thomas 1968). As a result of peroxidase activity (Bauch and Berndt 1973) they increase in size and become insoluble. A few studies have reported that relatively low molecular weight phenolics can inhibit enzymes per se (Firenzuoli et al. 1969, Nicholas and

Thomas 1968). However, phenolics that have been oxidised to their highly 'reactive' quinone derivatives are much more likely to prevent enzyme attack (Walker 1975, 1980). Quinone derivatives are capable of binding irreversibly to proteins and so denaturing them. Those reactions are mediated by phenol-oxidase enzymes, but phenol-oxidases have not been reported as being involved in pit membrane-enzyme interactions. Phenol-oxidases have been reported as being responsible for many of the oxidative browning reactions occurring in bacterially infected wood (Schink and Ward 1984). However, oxidative browning reactions are usually confined to the surfaces of wood because oxygen is necessary for the reaction.

The insolubility of high molecular weight polyphenolics precludes them from inhibiting enzymes unless they are first oxidised. Thus, the mechanism by which polyphenolics prevent the degradation of pit membranes in Douglas fir is most likely to be one of physical obstruction. Such a mechanism has been proposed to explain the resistance of encrusted cellulose microfibrils to cellulase attack (Nicholas and Thomas 1968, Green 1980). Tschernitz and Sachs (1975) noted that if polyphenolic compounds were present in the pit membranes of Douglas fir they did not interfere with any of the enzyme treatments they were using. Krahmer and Cote (1963) and Bailey (1966) found that heavy encrustations were not typical of Douglas fir pit membranes except in the ray tracheids and latewood, which, by implication, suggests that all other pits should be readily accessible to bacterial enzymes. It is possible that the pit membranes examined by various other workers

differ in their phenolic content. However, Bauch and Berndt (1973) commented that no differences in the amount of phenolic encrustations could be determined among different provenances of Douglas fir. There is evidence to suggest that the susceptibility of pit membranes to enzyme attack is different in air-dry and green timber (Bauch et al. 1973, Adolf 1975). As green wood dries, irreversible reactions between the phenolic components and cellulose occur which change the cell wall ultrastructure in a manner not completely understood. The wall and pit materials become less accessible to enzyme attack (Adolf 1975). Clearly there are some inconsistencies requiring further research.

At a macroscopic level, non-aspirated bordered pits present a large surface area for degradation because enzymes can gain access to both sides of the membrane. In contrast, the tracheid-to-ray, half-bordered pit membranes are accessible from one side only, at least until the ray parenchyma cells are breached. Parenchyma—to—parenchyma simple pits are even more inaccessible, bacteria must first enter the ray parenchyma before degradation can occur. Thus, bordered pits are degraded first, followed by half-bordered pits and finally by simple pits. The degradation sequences described in Section 4.5 (Figures 4-27 to 4-29) show that same pattern.

In summary, the chemical similarity of bordered pits, half-bordered pits and simple pits in Douglas fir suggests that they are all susceptible to attack by cellulase and pectinase enzymes. If polyphenolic encrustations are present in a membrane then they probably physically obstruct enzyme attack. Differing quantities of phenolic compounds on

the pit membranes may result in differential rates of enzyme attack, but that did not appear to be an important factor in this study. Furthermore apparent differences in susceptibility to enzyme degradation can be partly explained by differences in the amount of surface area available for enzyme-substrate interactions.

The fact that bacteria can improve the permeability and subsequent preservative treatability of wood in water-storage is not a novel idea. Most of the experiments reported in this study were designed to investigate the optimum requirements for successful permeability changes in sprinkled wood. Since permeability changes are mediated by bacteria the most obvious starting point was to examine the factors affecting bacterial growth.

There seemed little point in sprinkling logs and relying on naturally colonising bacteria to improve permeability. Instead logs were inoculated with bacteria known to improve preservative uptake. Further, it is important that those bacteria are competitive because it is impossible to exclude opportunistic colonisation from contaminating organisms during the sprinkling process. In one investigation into the sprinkling of Sitka spruce it was reported that few if any of the original species inoculated were isolated from the logs after as little as one week in storage (Fowlie 1981).

In any study which requires the repeated subculturing of bacteria onto solid or liquid media there are problems with maintaining the vigour of the bacterial population. Without continual testing it is difficult to know whether cultures retain the same identity or the same metabolic

capabilites after successive subculturing. With a mixed culture, as used in this study, those problems are magnified. One of the greatest dangers is that the growth of one or more organisms will proceed at the expense of others. A mixed culture was used in this study because the initial trials indicated that synergistic growth and enzyme production occurred in-vitro on pectic substrates. The relative importance of the five organisms was not determined. It was interesting to observe that the four genera of bacteria present in the mixed culture have been implicated in other studies as being capable of improving permeability (Ellwood and Ecklund 1959, Knuth and McCoy 1962, Macken and Pickaver 1979). It should be stressed that the mixed culture used in this study is not considered to be the most effective mixture of organisms available. In the context of this thesis it was not possible to conduct exhaustive tests to find and isolate the most effective organisms; that would have been a thesis in itself. The mixed culture performed adequately and no further screening seemed appropriate.

Although there are problems associated with using a mixed culture, particularly in an industrial situation, the obvious benefits conferred by the metabolic versatility of a number of different organisms outweigh those problems. That is especially true in a timber such as Douglas fir because the pit membranes have different compositions as discussed earlier. Unfortunately, it would be difficult to market a mixed culture and guarantee its composition.

Criticism could be levelled at the methods used to assess the size of the bacterial population in sprinkled

wood. Bacterial numbers were expressed as the number of bacteria per gram oven-dry weight of wood. Numbers were counted from dilution plates (Appendix M4) and therefore only bacteria capable of growing on the assay medium were actually counted. Problems associated with viable counts are well documented (Postgate 1969, Atlas 1982) but no other option seemed practical.

In previous studies (Ward and Fogarty 1973, Dunleavy and Fogarty 1971, Macken and Pickaver 1979) bacterial counts in water-stored wood have been presented as the number of organisms occurring per milliliter of sap squeezed from the sample. Carey 1979 suggested that an alternative method, which involved streaking small wood samples across agar plates, was less time consuming. A different approach was taken in this study; bacterial counts were expressed as the total number of organisms present per gram of homogenised wood tissue. Schink et al. (1981(a) and (b)) used a similar technique in an investigation into the microbiology of wetwood. Counts of bacteria per millilitre of sap tend to under-estimate the total number of bacteria present. Bacteria generally do not exist suspended in a fluid unless that fluid is constantly being agitated; in most circumstances they are attached to surfaces (Costerton and Cheng 1982). Consequently, it is likely that some bacteria will remain inside a wood sample when the sap is squeezed from it. Bacterial cells may also have remained lodged in the macerated wood samples used in this study, under-estimating the number of bacteria present. It is evident that there are limitations to any technique for counting bacteria and all are subject to a large margin of

error. For the purposes of this study relative, not actual numbers of bacteria are important.

The bacterial counts reported in Sections 4.4.4 and 4.5.4 are a measure of the total number of bacteria present on the dilution plates, but no attempt was made to separate the total count into counts of individual species. There were a number of reasons for this, the major one being that the five organisms making up the mixed culture were not readily distinguishable as young colonies on agar plates. Identification required sampling each colony, making a smear, staining and then examining the stained preparation under a microscope - a time consuming process. Even then gram staining produced variable results and the Bacillus spp. did not form spores readily, precluding their identification on that basis.

Some measure of the population dynamics would have provided an interesting insight into the successional changes and synergistic activity at different stages during the sprinkling process. Such an investigation was carried out by Macken and Pickaver (1979) in water-stored Sitka spruce. They determined that there was a progressive decrease in the number of bacterial species isolated from water-stored wood as the length of storage time increased. There was a change from aerobic bacteria to facultative anaerobes during the storage period and also a change in the enzyme capabilities of the isolated organisms. No pectinase producers were isolated until after three weeks' storage. In this study Bacillus spp. and an Enterobacter sp. could be isolated at the end of seven to eight weeks' sprinkling treatment indicating at least that

the organisms were competitive.

The distribution of bacteria throughout woody tissues is just as important as the total number present. Distribution is affected by the ability of bacteria to move inside wood; a phenomenon primarily dependent on the anatomy of the wood under investigation. Bacteria must disseminate rapidly into timber if short sprinkling times are to be realised. There is evidence to suggest that much of the uneven preservative distribution found in water-stored timber is a result of the uneven distribution of bacteria in wood during storage (Ellwood and Ecklund 1959, Knuth and McCoy 1962, Bauch et al. 1973)

In the intial stages of colonisation, bacteria probably enter a large log radially rather than axially because of the distances involved. However, once inside the wood, axial migration is liable to be much faster than tangential or radial migration. To avoid preferential axial entry in the short bolts used for the sprinkling trials the exposed axial faces were end-sealed.

There were indications from the preliminary trials reported in Chapter three that bacteria migrated most readily in the tangential direction, presumably via bordered pits. In normal, undamaged roundwood poles there is little opportunity for bacteria to enter wood tangentiallly. The purpose of incising the quartered bolts as described in Section 3.3 was to allow bacteria tangential access to the green wood at least to the depth of the incision. However, any improvement in eventual preservative penetration as a result of surface incising was confounded by the fact that the sawn faces of the timber also acted as deep

incisions. More useful results were obtained using incised roundwood; incising increased the radial depth of preservative penetration presumably because bacteria had better access to the wood tissues.

Bacteria can enter wood using radial or tangential pathways. To determine which pathway was most efficient, measurements of radial and tangential migration were made in kerfed and non-kerfed material (Chapter four). The single kerf provided a tangential point of access and it was possible to map tangential migration of bacteria around the stem using the kerf as a point of origin. The results (Section 4.4.4) indicated that the tangential distance moved by bacteria was approximately linear with time. One interesting aspect of the data was that the rate of migration appeared independent of the nutrient treatment suggesting that wood anatomy was the major limiting factor. The sampling method used to measure radial migration was too insensitive to demonstrate a quantitative relationship of migration with time. However, the data in Section 4.5.4 indicate that radial migration into the inner sap (a distance of approximately 1.5cm) was slow. Although it is not possible to compare radial and tangential migration in terms of absolute velocities, it can be inferred from the relative magnitudes of the distances involved that tangential migration is much faster. That result is to be expected in Douglas fir because the ray tissues are so impermeable. The opposite situation is reported for pine species. It is generally accepted that in pines the ray tissues are preferentially colonised and that the rays provide the most rapid means of bacterial entry

(Ellwood and Ecklund 1959, Unligil 1969).

Tangential migration of bacteria into Douglas fir should increase the rate of permeability improvement and that permeability should be more uniform around the stem. It is possible to predict a minimum time requirement for the uniform spread of bacteria around a roundwood stem. In a practical situation, if bacteria are provided with some means of tangential access to roundwood then they will use both radial and tangential pathways to colonise the wood. It was never intended that a single kerf provide the only tangential access into poles in a full scale sprinkling trial. Uniform spread of bacteria from one kerf would be too slow for the process to economic. Nevertheless, the results presented in Section 4.4.4 suggest that bacteria would have eventually migrated to all areas of the sapwood had the experiment been left longer. Assuming that bacteria would have been uniformly distributed from a single kerf after ten weeks sprinkling, then by implication, if five equally spaced kerfs or deep incisions had been available, the same result would have been achieved in only two weeks. Of course in poles with a diameter greater than 150-200mm more incisions will be necessary. The theory is also a little simplistic because not only do bacteria have to migrate to all areas in the sapwood but they also need sufficient time to produce the required enzymes to degrade the pit membranes enough to improve permeability, a point which will be discussed shortly.

Bacterial movement in wood is poorly understood, yet the phenomenon is of fundamental importance to this study. Two types of movement can be visualised, microscopic

movement within individual wood cells and macroscopic movement between cells. The extent of current knowledge on bacterial movement in wood was reviewed in Chapter three. Bacteria are thought to pass from cell to cell through the pit membranes (Harmsen and Nissen 1965, Boutelje and Bravery 1968, Greaves 1970(b)). Bacterial cells are generally much larger than the pores present in pit membranes and therefore they must 'eat' their way through by degrading each pit membrane sufficiently to permit free passage. The openings in pit membranes must be considerably larger than the bacterial cells otherwise blockages will occur. Consequently, migration from cell to cell must be a slow process very much dependent on wood anatomy. Several reports support that contention. Liese and Karnop (1968) found that bacteria had migrated to the heart-sap boundary in spruce after 12-16 weeks water storage. The same feat was accomplished in only four weeks with pine although in both instances the authors gave no indication of the width of the sapwood band. The difference can most likely be explained by the greater permeability of the ray tissues in pine. Macken and Pickaver (1979) followed the migration of bacteria into water-stored Sitka spruce over a twelve week period. They observed that bacteria had penetrated to the inner sapwood by the fourth week of storage. At the end of the seventh week, bacteria were evenly distributed over the entire sapwood zone.

So far the pathways for bacterial movement have been considered. Another issue to examine is whether bacteria move actively or passively along those pathways. Greaves (1973) reported that bacterial motility was not a limiting

factor in the decay of small (2.5mm wide) Eucalyptus regnans (F. Muell) samples. After several weeks incubation with both motile and non-motile bacteria, strength losses were found to be similar although he suggested that the result was due to the small size of the specimens. Greaves (1971(a)) speculated that bacteria could move across cell surfaces by cell division. Greaves calculated that ten tracheid widths or ten parenchyma cell lengths could be traversed in a 24 hour period. It would appear that he used cell replication times of around twenty minutes for that calculation. It is most unlikely that such short replication times are achieved by bacteria inside wood under water-storage. The pit membranes must also be completely unobstructed for such rapid movement to occur. In an unrelated investigation into the ability of bacteria to penetrate porous sandstone, Jennerman et al. (1985) determined that motile bacteria migrated through sandstone cores at a rate three to eight times faster than non-motile bacteria. Wood may also be regarded as a porous medium and it is possible that motile bacteria behave similarly in both. Although hard to imagine, Jennerman et al. (1985) postulated that non-motile bacteria migrated by filamentous division (Greaves (1971(a)) had a similar idea). An alternative suggestion for the movement of non-motile organisms was that the bacteria could be driven through porous sandstone as a result of gas pressure generated by metabolic processes (Jennerman et al. (1985)).

Bacterial motility would be efficient in penetrating wood only if it was largely unidirectional. Shoesmith (1960) observed the unidirectional movement of Bacillus

brevis in-vitro at speeds which ranged from 6 to 32 µm per second with the majority of cells achieving 25 µm per second. Thus, under ideal conditions some bacteria have the capacity to move large distances (relative to their size) in short periods of time. Motility may be directional in response to tactic stimuli (e.g. to dissolved oxygen concentration) (Baracchini and Sherris 1959, Adler and Dahl 1967). Although the evidence presented is not definitive proof, the possibility that 'self' motility allows bacteria to move through wood is highly likely. All of the organisms present in the mixed inoculum used in this study were found to be motile in-vitro. Even if motility is of minimal importance to those bacteria, it is difficult to imagine that it could be a disadvantage.

There have also been suggestions that bacteria inside wood are carried along by water currents (Greaves 1970(b)). It has been suggested that currents deep inside wood are created when partial drying is followed by alternate rewetting and drying of the sample surface. When the surface of a softwood timber is partially dry, water is under tension because liquid menisci form in the exposed tracheid ends. When the timber is rewetted the menisci collapse and suck fluid in (Tisseverasinghe 1975). Such a mechanism is plausible with sawn timber when a large number of tracheids are exposed, but it is difficult to imagine that the same process would work efficiently in roundwood without some form of modification (such as incising). A comparison of the effect of cyclic sprinkling on incised and non-incised quartered Douglas fir bolts was made in the preliminary trials outlined in Chapter three. The results

were inconclusive and the trials were discontinued.

The simplest method of optimising bacterial growth in wood under water-storage was to provide the colonising organisms with an adequate nutrient supply. The concentration of nitrogen in wood and the pH of the cell sap were thought to be sub-optimal for bacterial growth. It is generally accepted that the low quantities of nitrogen present in wood limit fungal decay (Cowling 1963). Therefore it seemed reasonable to assume that nitrogen might also be a limiting factor for bacterial growth and metabolism inside logs under water-storage. To examine the validity of that assumption, nitrogen in the form of ammonium sulphate was added to some of the sprinkling solutions. Comparisons of bacterial growth, enzyme production and subsequent preservative uptake were made between logs sprinkled with and without added nitrogen.

Acidic conditions are generally considered unfavourable for bacterial growth. Frequently bacteria lower the pH of their environment by oxidising available sugars to organic acids. Phosphates are often incorporated into microbiological culture media to counteract acid formation and also to provide a source of phosphate for general bacterial metabolism (Pirt 1975). Due to the inherent low pH of Douglas fir sap and the presence of oxidisable sugars (Section 3.3, Chapter three), potassium phosphate was added to some of the sprinkling solutions.

It would have been interesting to examine the effects of different concentrations of nitrogen and phosphate on bacterial metabolism. Unfortunately, because of the experimental design, comparisons are limited to the effects

of the presence or absence of the two nutrients. Ideally, the logs should have been uniformly impregnated with the nutrient under test at the start of the experiments. However, it would have been impossible to achieve uniform impregnation without also modifying other factors inside the wood. For example, drying the timber and then treating with a nutrient solution under a vacuum-pressure procedure would not have been practical with Douglas fir because of its impermeability. Such a procedure would also have resulted in unacceptable changes to the wood's chemical composition (Adolf 1975). Some measure of nutrient impregnation might have been achieved by adapting the oscillating pressure method (McQuire 1964). However, that method would have removed most of the gases present in the wood and replaced them with nutrient solution. The presence and composition of the gases in wood may have an important influence on bacterial metabolism. It is apparent that any method to achieve uniform impregnation of the nutrients before the sprinkling treatment began would have resulted in substantial changes to the wood as a substrate.

Consequently, nitrogen and phosphate were added with the sprinkling solution and as sprinkling progressed they diffused slowly into the wood. A comparison of the data presented in Sections 4.4.2 and 4.5.2 suggests that the rate of diffusion in a tangential direction was greater than that in a radial direction. That finding is supported by Burr and Stamm (1947) who showed that the ratio of radial to tangential diffusion of ferric chloride in Douglas fir sapwood was less than unity. In most timbers radial diffusion is faster than tangential diffusion (Burr and

Stamm 1947, Becker 1976) because the ray tissues provide an excellent pathway for the radial diffusion of solutes. Comparisons of the diffusional rates of potassium phosphate and ammonium sulphate were not quantitative.

Christensen (1951) found that solutes containing either a bivalent anion or cation diffused into wood slower than univalent electrolytes. Christensen suggested that wood has a tendency to act as a negatively charged semi-permeable membrane. Thus it can be expected that the diffusion rates of potassium phosphate and ammonium sulphate into Douglas fir are different.

Chapter three examined the influence of nutrient concentrations on enzyme production and bacterial growth in-vitro, but it is difficult to relate the results of those trials to the sprinkling experiments because nutrient gradients were formed in sprinkled wood. The results serve to show only the effect of the presence or absence of the nutrients.

Nutrients added to sprinkled wood can affect the resident bacterial population in two ways: by stimulating bacterial growth or by stimulating enzyme production. The data presented in Section 4.4.4 indicated that phosphate buffer alone or in combination with nitrogen stimulated bacterial growth in the outer sap regions for the first three weeks of sprinkling. Thereafter the presence or absence of buffer had no effect on bacterial numbers. It appears that phosphate buffer effectively shortens the lag period in bacterial growth. However, the presence or absence of nitrogen alone had little effect on bacterial growth at any time; a surprising result, but one which may be

explained by the ability of Bacillus polymyxa to fix nitrogen under anaerobic conditions (Hino and Wilson 1958, Grau and Wilson 1962, Buchanan and Gibbons 1975).

Few studies have examined the percentage composition of gases in water-stored wood. Hayward (1981) found that the composition of gases in Pinus radiata logs stored under sprinklers for four years was similar to that reported by Thacker and Good (1952) in standing timber. The composition of gases present in standing timber is different to gaseous mix in the atmosphere. The concentration of carbon dioxide may be up to several hundreds of times higher and the concentration of oxygen 25% lower than that in the atmosphere (Thacker and Good 1952, Jensen 1967). The proportion of nitrogen is reported to be similar to that in air (Chase 1934). Thus nitrogen gas is potentially available for fixation by bacteria. Evidence from studies of bacterial succession in water-stored logs indicates that in the early stages of storage aerobic conditions prevail but after extended storage anaerobiosis occurs (Macken and Pickaver 1979). The change is presumably due to bacterial consumption of oxygen and, in addition, the gases are replaced with liquid as the wood becomes more saturated. The data presented in Section 4.4.1, Chapter four, showed that the percentage moisture saturation in freshly felled timber increased from 83% to nearly 100% of the theoretical maximum after four weeks sprinkling. The change from aerobic to anaerobic conditions is likely to be more pronounced in ponded wood than sprinkled wood, but to the author's knowledge that has not been investigated. A sprinkling solution is constantly being aerated, but the influence of

such aerated water on the oxygen concentration deep inside the timber may not be of much consequence. Huang et al. (1977) noted that the oxygen diffusivity in liquid saturated Douglas fir sapwood was low.

The effect of nutrients on bacterial enzymes is more complex because not only is enzyme production affected, but the conditions inside the wood controlling enzyme activity are also involved. Fernando (1937) noted that the principal factor controlling the production of pectinases by Bacillus subtilis and B. carotovorus was the pH of the growth medium. Fernando noted that a low C:N ratio in the growth medium resulted in a low pH and a consequent reduction in pectinase production. He found that if media were buffered between pH 5.5 and pH 8.5, enzyme production was similar over a wide range of C:N ratios. The pH changes in sprinkled wood closely paralleled potassium phosphate diffusion into the tissues (Section 4.4.3). Consequently in those bolts sprinkled with phosphate buffer, pH gradients extended from the outer sap to the inner sap.

Production of polygalacturonate transeliminase (PGTE) in the outer sap of kerfed Douglas fir was highest in those bolts sprinkled with a combination of nitrogen and phosphate, followed by phosphate alone and then nitrogen alone (Section 4.4.6). Without added phosphate or nitrogen PGTE production was low. Unfortunately, it is difficult to establish a direct relationship between PGTE production and the pH of the sap. Similar pH values were recorded in all treatments containing phosphate buffer but enzyme production was different. The pH in the nitrogen treatment was similar to that in the water treatment but more enzyme was produced

in the presence of nitrogen. Clearly some factor other than pH may also be involved in PGTE production. The influence of pH on enzyme activity in the sap is probably of greater importance. Nagel and Vaughn (1961) found that the pectate enzymes produced by Bacillus polymyxa were rapidly inactivated at pH values below pH 5. Optimum activity occurred in alkaline conditions between pH 8 and pH 9. The enzymes assayed from sprinkled Douglas fir also showed optimum activity between pH 8 and pH 9 (Section 4.4.6). Thus, sap pH conditions in buffered sprinkling treatments are more likely to favour PGTE activity which should lead to more rapid degradation of pit membranes.

Peak PGTE production was detected after four weeks sprinkling in all treatments. Comparisons of PGTE production after four weeks sprinkling revealed that the high enzyme concentration in the nitrogen and phosphate treatment was due to a greater production of enzyme per bacterial cell. It was interesting to observe that after four weeks sprinkling the concentration of enzyme present in the sap fell at the same time as the bacterial population declined. The result suggests that enzyme production is growth linked and occurs mainly during the logarithmic phase (up to four weeks in the outer sap under the conditions used in this study). In-vitro production of pectic enzymes by Bacillus polymyxa also occurs during logarithmic growth (Nagel and Vaughn 1961,1962).

The preliminary trials reported in Chapter three showed that PGTE production in the mixed culture was induced by the presence of polygalacturonate in the culture medium. Small amounts of PGTE were produced constitutively

with glucose as the sole carbon source but the results were variable. The possession of inducible enzymes is an advantage to bacteria because it enables the organisms to conserve energy by not producing enzyme proteins unless degradable substrates are present. When the substrates are available they are degraded by the constitutive enzymes which release breakdown products capable of inducing further enzyme production. A disadvantage of induced enzyme synthesis in terms of optimising permeability improvement is that there is an inevitable time lag before significant quantities of enzyme are produced. The evidence presented in Section 4.4.5 seemed to indicate that PGTE production lagged behind bacterial growth but the observation could not be quantified.

There was no evidence to indicate that extensive enzyme diffusion occurred; enzyme activity was only detected where bacteria were present. Poor diffusion of enzymes may be explained by the high molecular weight recorded for some bacterial lyases (Lubomira and Markovic 1976) and the ability of wood to influence the migration of charged molecules. Courtois (1966) noted that bacteria needed to be in close contact with the cell wall for cellulose degradation to occur. Tschernitz (1973) found that 'pectinol', a commercially available pectinase of fungal origin, was able to diffuse radially into green Douglas fir sapwood wedges quite readily. Within three weeks the enzyme was evenly dispersed around the entire sapwood. It would be of interest to know the molecular weight of his enzyme to make comparisons with the observations in this study. In contrast, Meyer (1974) pre-treated Douglas fir sapwood with

another commercial pectinase and found that the resulting permeability increases were not uniform all over the sample. Meyer attributed that variability in part to the poor penetration of enzyme into the wood during the pre-treatment procedure.

It was noted in Chapter four that a number of problems occurred with the enzyme analyses. Pectinase activity in squeezed sap from the outer sap of non-kerfed Douglas fir (Section 4.5.5) decreased with storage at room temperature. Those losses were attributed to oxidative browning reactions which took place when sap was exposed to air for any length of time. There was no evidence to indicate that the browning reactions took place inside the wood tissue. Therefore the problem is related to the enzyme assay only. It is unlikely that prolonged freezing decreased enzyme activity significantly. Nagel and Vaughn (1961) found that frozen pectinase extracts from Bacillus polymyxa were stable for long periods of time.

There have been few investigations into the effects of nutrient supplements on permeability changes in water-stored wood. Fogarty (1973) found that carbon was a limiting factor to bacterial growth in water extracts of Sitka spruce saw dust and that adequate amounts of nitrogen and trace elements were present in sapwood with nutrient supplements. Johnson (1979) examined the effect of nutrient broth and glucose on the degradation of pit membranes in microtome sections of Abies grandis sapwood. Contrary to his expectations the greatest degradation occurred in the presence of nutrients. In the same paper Johnson states that the addition of nutrients to large billets did not result in

any permeability improvement after eight weeks incubation with Bacillus polymyxa. However, if the data he presents for Douglas fir after four weeks incubation are re-examined, it is apparent that the permeability in samples supplemented with nutrients is three times higher than that in samples without nutrients. The improvement in permeability from four to eight weeks is small suggesting maximum permeability change occurred in the first four weeks. It is possible that some stimulation of bacterial growth in sprinkled Douglas fir could be achieved if an external carbon source was added to the sprinkling medium, but the possibility was not examined here.

The difference between the amount of preservative uptake in non-sprinkled bolts and that in sprinkled bolts indicated the success of the bacterial treatments. Other studies have relied on dip tests and permeability measurements in small samples to provide an indication of permeability changes in water-stored wood (Greaves 1970(b), Hayward 1981). There are indications that the permeability of small samples is often not indicative of the eventual preservative uptake in whole logs (Booker 1980(a)). Measurement of the preservative uptake in whole logs has its own problems, not least of those being variability. The size of the test specimens used in this study was a compromise between space requirements and statistical replication. To counter any 'end' effects, the short test bolts used in the sprinkling treatment were end-sealed with epoxy-resin. Failure of the end-seal occur in some instances with catastrophic results. The entire sapwood band and a proportion of the heartwood became

impregnated with preservative regardless of the permeability of the sample. Despite those difficulties it was still possible to detect differences in preservative uptake in the different treatments under investigation. At this point it should also be stressed that the preservative uptakes obtained with end-sealed bolts were due to radial uptake only in non-kerfed bolts or to radial and tangential uptake in kerfed bolts. In an industrial situation the wood would not be end-sealed and uptake could be expected to be improved by the additional axial movement of preservative into the tissues.

The temperature during sprinkling had a pronounced effect on preservative uptake. The preservative uptake in quartered bolts sprinkled for eight weeks at a temperature of 23-24 °C was higher than that for similar bolts sprinkled at 13-14 °C. The improvement in uptake was attributed to one or more of the following: better bacterial growth, increased rates of nutrient and enzyme diffusion and an increased rate of enzyme activity although none of those parameters were measured. It is well known that seasonal temperature during water-storage affects permeability improvement (Lutz et al. 1966, Liese and Karnop 1968, Boutelje 1976). Banks (1970) investigated the effect of temperature on permeability changes in Scots pine submerged in small tanks. Banks found that logs stored at 20 °C showed a greater improvement in permeability than logs stored at 10°C. Storage at 30°C did not result in any significant improvement in permeability over and above that occurring at 20 °C.

The results presented in Section 4.4.6 showed that

sprinkling improved preservative uptake in kerfed bolts. However, the increase in uptake was not linear with time and there were differences among the four nutrient treatments. Preservative uptake doubled in all treatments in the first week of sprinkling. From week 1 to week 4 the uptake in treatments without nitrogen remained constant while the uptakes in treatments containing nitrogen fell. From week 5 onwards the uptake in all treatments rose and ultimately at the end of seven weeks sprinkling the highest uptake was observed in the treatment containing nitrogen and phosphate. Regression analysis of uptake with radial and tangential preservative penetration showed that the changes in uptake were more closely related to the depth of tangential penetration rather than to radial penetration. The temporary decrease in uptake observed in treatments which contained ammonium sulphate was puzzling. The possibility that bacterial cells were blocking the pathways for preservative entry was investigated but a statistical relationship was not found.

Sprinkling also improved the preservative uptake in non-kerfed bolts. Comparisons of uptake in kerfed and non-kerfed wood are confounded by end-seal failure and checking in the latter case. At the end of eight weeks sprinkling total sapwood preservative uptake was observed in non-kerfed wood contrary to expectations from earlier experiments. The effects of the different nutrient regimes on preservative uptake were similar to that in kerfed material because of a similar influence on bacterial growth. Interestingly there was no significant fall in preservative uptake from two to four weeks' sprinkling, yet

polysaccharide 'slimes' were observed in the tissues. The observation cannot be explained with the available data. A repeat of the experiment taking greater care to prevent end-seal failure and checking during drying would be useful.

Scanning electron micrographs of wood samples removed from close to the kerf after three weeks sprinkling with nitrogen and phosphate indicated the presence of amorphous and fibrillar substances coating the bordered pits and the surfaces of the ray cells (Figure 4-29). Superficially, the fibrillar web evident in the micrographs resembles those described by Butterfield and Meylan (1973), covering the vessel members of Coriaria arborea Lindsay. However in that instance the microfibrillar web was almost certainly of plant origin. The composition of the material seen in Figure 4-29 is unknown, but it is probably polysaccharide 'slime' of bacterial origin. Martin et al. (1968) examined the 'slime' produced by Cytophaga johnsonii grown aerobically in a liquid glucose medium. Transmission electron microscopy of the 'slime' revealed that it consisted of a densely interwoven network of filaments surrounded by matrix materials; an appropriate description of the material observed in sprinkled Douglas fir. The appearance of the material was very much dependent on the sample preparation procedure, but from the two examples given in Figure 4-29 it seems plausible that such a coating could reduce permeability. It was interesting that no fibrillar material was present on samples removed from next to the kerf after sprinkling for five weeks or more.

Bacillus polymyxa is known for its ability to produce copious amounts of extracellular polysaccharide. The Greek

prefix (poly-) means much and (-myxa) means 'slime' or 'mucus'. Hence, polymyxa means much 'slime' (Buchanan and Gibbons 1975). Grau and Wilson (1962) noted that B. polymyxa produced copious mucilaginous 'slime' when grown aerobically on sucrose. Slime production was associated with the logarithmic growth phase. Martin et al. (1968) also noted that 'slime' production ceased at the end of logarithmic growth and they also suggested that the 'slime' was enzymatically degraded during the stationary phase of growth.

A number of investigations have noted that permeability increases during water-storage are not linear with time (Bauch et al. 1970, DeGroot and Scheld 1973). Other studies have attributed reductions in preservative uptake and retention to the production of polysaccharide 'slimes' by colonising bacteria (Greaves and Barnacle 1970, Banks 1970, Greaves 1971(a), Banks and Dearling 1973). Greaves and Barnacle (1970) found that the preservative retention in water-stored slash pine (Pinus elliottii) was highest in the sapwood at the heart-sap boundary. They suggested that the phenomenon could be explained by differences in the amounts of bacterial 'slimes' present in the inner and outer sapwood. More 'slime' was present in the outer sapwood because nutrient substances were more available to the bacteria and consequently preservative retention was lower.

It is interesting to speculate on the possible mechanism by which bacteria and 'slimes' decrease permeability. During the logarithmic growth phase, bacteria produce pectinase enzymes which degrade the pit membranes,

theoretically improving permeability. However, it appears that the bacteria also produce copious amounts of extracellular polysaccharide material during their logarithmic growth phase. When wood is dried prior to preservative impregnation, the polysaccharide material and associated bacterial cells form a film over the cell walls and the partially degraded pit membranes and the permeability is actually reduced.

There are two likely explanations for the improvement in preservative uptake observed after five weeks sprinkling. When the pit membranes reach a certain stage of degradation, drying stresses cause the small holes to coalesce and the membranes rupture. The coating of 'slime' is broken at the same time and consequently the permeability is improved. Alternatively as soon as the logarithmic growth phase ceases 'slime' production also stops and any existing 'slime' is degraded enzymatically.

If 'slime' production is highest under anaerobic conditions then perhaps the increase in permeability after five weeks sprinkling is because conditions become anaerobic as the bacteria move deeper into the wood. 'Slime' production slows as the oxygen concentration falls and so permeability improves. All of published reports examined note that B. polymyxa produced 'slime' under aerobic conditions but they do not indicate whether the 'slime' is also produced in anaerobic conditions.

The permeability of green wood as well as dry wood is affected by sprinkling. Measurements of tangential permeability in green sapwood (Section 4.7.2) showed that permeability close to the kerf was half that of samples

taken from deeper inside the wood. One plausible explanation for the result is that bacteria are more numerous close to the kerf and their presence obstructs liquid flow.

Although the addition of potassium phosphate to the sprinkling solution improved preservative uptake, the results presented in Section 4.4.6 indicate that phosphate affected the distribution of copper in preservative treated wood. Low levels of copper were correlated with the presence of potassium phosphate. The result has important ramifications because treated wood deficient in copper is susceptible to premature decay. Phosphate may interfere with the initial cationic fixation of copper to the wood substrate; the relative concentrations of K+ ions and Cu++ ions present will determine the degree of interference. It may be necessary to study copper fixation as a function of potassium phosphate concentration to confirm that hypothesis. There are indications in the literature that inorganic salts interfere with copper in CCA treated wood. Plackett (1984) found that inorganic salt solutions enhanced leaching of copper from CCA treated Pinus radiata posts.

The unified concept of permeability, drying and preservative uptake has already been discussed in Chapters two and five. It is not surprising then, that the effects of sprinkling on drying and preservative uptake were found to be similar. In the first four weeks of sprinkling the radial and tangential drying rates of cubes cut from sprinkled bolts fell below the corresponding rates for non-sprinkled cubes. The lowest drying rate occurred at four weeks. From five weeks onwards radial and tangential drying rates

improved and were higher than the rates for non-sprinkled material at the end of eight weeks sprinkling. The two treatments without phosphate buffer improved the drying rate most of all.

The improvement in drying rate is likely to be the result of pit degradation and a consequent improvement in moisture flow. The fall in drying rate up until four weeks sprinkling is probably due to bacterial 'slime' obstructing moisture flow paths as already described for preservative uptake. Thus, the length of sprinkling time appears to have a direct effect on drying rate. Boutelje (1977) and Boutelje et al. (1978) formed similar conclusions after drying spruce and pine ponded for different lengths of time. In Chapter five it was suggested that there are conflicting reports on the effects of water-storage on drying; the confusion is partly because of the influence of storage time but also because a variety of species have been examined.

The pathways for radial and tangential movement of water in non-sprinkled wood during drying were originally believed to be different. Tangential movement of water was thought to take place via the bordered pits in the tracheids; radial movement was thought to occur through the rays. The relative permeability of the two tissues suggested that tangential drying would be faster. In fact the results presented in Section 5.3 indicate that the rate of radial drying in non-sprinkled wood slightly exceeds that of tangential drying. The petroleum jelly end-sealant did not fail suggesting that the ray tissues might be permeable. However, that explanation is unlikely because of the anatomical characteristics of Douglas fir rays. A more

plausible hypothesis involves the function of tracheid bordered pits during radial drying.

In the accepted stylised concept of softwood structure, all tracheids are orientated so that the bordered pits face in a tangential direction. A few bordered pits face in a radial direction at earlywood-latewood boundaries but generally the bordered pits are not believed to function efficiently in radial movement of fluids. The results Section 5.3 show that sprinkling improves both the radial and tangential drying rates in Douglas fir in a similar manner. The improvement in tangential drying is explained by the destruction of bordered pits but improvements in radial drying cannot be explained by changes in the ray pitting. The most obvious conclusion therefore is that radial and tangential moisture flow must occur through bordered pits. Great care was taken to ensure that the faces of each cube used for the directional drying experiments were orientated correctly. Therefore, the phenomenon can only be explained by irregularities in the wood structure. That is, an appreciable number of bordered pits must be orientated so that they can contribute to radial flow. Evidence to support such an argument comes from the fact that the radial drying rate in non-sprinkled, resaturated wood was retarded. The reduction in the drying rate of resaturated wood is usually attributed to bordered pit aspiration. Both radial and tangential drying rates in sprinkled, resaturated wood are improved. That can be attributed to damage to the bordered pits preventing effective pit aspiration which by implication means that radial drying occurs at least partly through bordered pits.

It is unlikely that the ray tracheids in Douglas fir account for the radial drying rates observed because ray tracheids are so uncommon. The ray tracheid bordered pits are also frequently too encrusted to conduct fluids efficiently. One other pathway potentially capable of contributing to radial drying is the interstitial space system between adjacent ray cells illustrated in Figure 4-29. Interstitial spaces may conduct fluids during drying if the so called 'blind' pits which connect the spaces to the adjacent cells are permeable. However, their appearance in scanning electron micrographs makes that possibility unikely. Furthermore, there was no indication of any bacterial attack on the 'blind' pits at least within the time span of the sprinkling experiments. There has been some speculation in the literature that interstitial spaces are important for the transport of gases to respiring cells (Back 1969, Bolton et al. 1975), but such a role has not been proved.

Although such a simple cause and effect hypothesis explains the effect of sprinkling on drying, the effect of sprinkling on shrinkage properties is not so easily explained. The data in Section 5.2 indicate quite conclusively that the bacteria invading wood during sprinkling, reduce tangential shrinkage. Reduced tangential shrinkage was manifest as a reduction in kerf opening and also by smaller dimensional changes in cubes cut from sprinkled wood. The result cannot be explained by differences in the equilibrium moisture content between sprinkled and non-sprinkled wood and 'bulking' by the nutrient salts solution is not a feasible option. Bulking

should have affected radial shrinkage as well as tangential shrinkage but it did not. In fact, tangential shrinkage was reduced even when nutrient salts were not added to the sprinkling solution. The following hypothesis offers a possible explanation for the shrinkage phenomenon.

In saturated cell walls, water occupies the void space between adjacent cellulose microfibrils and the hemicellulose 'packing' materials. The water molecules are believed to be hydrogen bonded to the abundant, exposed hydroxyl groups on the cellulose and hemicellulose polymers (Skarr 1972). When wood is dried below fibre saturation point the water molecules are driven off allowing the hydrogen bonds between the carbohydrate polymers to reorganise. Reorganisation results in the void spaces becoming smaller and the phenomenon is manifest as cell wall shrinkage. Greaves (1973) has suggested that bacteria invading wood tissues are able to affect the microstructure of the cell wall through enzyme attack. Greaves postulated that bacterial enzymes selectively remove the 'packing' materials between the cellulose microfibrils which causes a reorganisation of hydrogen bonding; the cell wall consequently becomes more crystalline and brittle. Barber and Meylan (1964) and Cave (1972) have suggested that the microfibrillar organisation of a cell wall determines its shrinkage characteristics. Hence if Greave's theory is correct then cell wall shrinkage may well be affected by bacteria. Theoretically, removal of the cell wall matrix materials should result in an increase in the size of the void spaces between fibrils, allowing more space for water molecules. If that occurred, the cell wall might be expected

to expand. However,there was no evidence that the kerf closed up during sprinkling which might be expected if the cell walls expanded.

It could be argued that radial shrinkage should be affected by bacterial action in a similar manner to tangential shrinkage; the results of this study suggest that it is not. Two hypotheses can be put forward to explain why radial shrinkage is not affected. The first may be visualised as the reverse of the 'ray restraint' theory in which the ray tissues are supposed to restrain radial shrinkage. The second is more simplistic and relies on the fact that the width of tracheid cells is smaller in a radial direction than it is tangentially. Consequently, less wall material is able to be attacked by bacterial enzymes and any alteration in radial shrinkage is reduced.

The literature surveyed on the water-storage of wood makes no mention of its influence on shrinkage properties. However, Boutelje (1976) and Boutelje and Ihlstedt (1978) reported that the tendency of water-stored Scots pine and Norway spruce to check was slightly higher than that in freshly felled wood. Boutelje (1976) observed that sprinkling and ponding both caused wood to check but ponded wood showed greater permeability. Boutelje speculated on the possibility that different forms of enzyme attack were responsible for the two observations.

Finally some comment should be made about the discolouration observed in sprinkled wood after drying. In Figure 4-32 it was evident that a reddish-brown stain developed on the exposed surfaces of sprinkled bolts. In the longitudinal direction, the stain was limited to a shallow

(1-3mm) zone adjacent to the exposed surface. As the length of sprinkling time increased the stain migrated towards the heartwood in non-kerfed bolts and away from the kerf in kerfed bolts, suggesting that the staining was related to the presence of bacteria. Figure 4-32 also showed that saprophytic fungi colonised those areas that were not stained. The fungi may be inhibited by the presence of bacteria or they may be inhibited by compounds formed during the browning reactions.

Brown staining in wood during drying is a well known phenomenon which is not fully understood. Miller et al. (1983) found that orange and brown stains developed on Douglas fir planks sprayed with water continuously for less than a week. Stain development was enhanced in warm humid conditions and was visible up to one inch into the timber. Miller et al. were able to show that the stains developed in partially anaerobic conditions suggesting that non-oxidation reactions were involved. They were unable to determine if bacteria caused the staining but the evidence indicated that enzymatically catalysed reactions were responsible. Steaming seemed to prevent staining, possibly because it denatured the enzymes involved.

CHAPTER 7

SUMMARY AND CONCLUSIONS

The sapwood and heartwood of New Zealand grown Douglas fir are impermeable to water-borne preservatives because of the wood's anatomical characteristics. The permeability and preservative uptake of sapwood can be improved by bacteria during storage under water sprinklers, but no effect was observed in heartwood. Improvements in permeability were related to the enzymatic degradation of pit membranes.

Ideally, to be able to use Douglas fir roundwood in high decay hazard environments, sapwood must be completely impregnated with preservative. Bacteria mediate permeability changes, so the time taken to achieve total sapwood preservative penetration is influenced by how readily bacteria can migrate into and through the timber.

In roundwood poles, bacteria can enter the woody tissues only along axial or radial pathways. It is difficult to imagine that axial entry into a whole log is significant because of the distances involved. In this study experiments showed that tangential movement of bacteria was considerably more rapid than radial movement. Therefore, the effects of incising and kerfing on bacterial entry into wood were examined and it was found that incising provided an ideal pathway for tangential entry into wood; not only was entry more rapid from a kerf or incision but the spread of bacteria throughout the tissues

was more uniform. Tangential migration from a single kerf was approximately linear with time and it was possible to predict the minimum number of kerfs/incisions necessary to achieve a uniform distribution of bacteria around the entire sapwood zone.

The conditions within the wood for bacterial growth and metabolism are also important. An increase in temperature from 13°C to 23°C increased subsequent preservative uptake in quartered bolts by up to 40%. Nutrient supplements added to the sprinkling solutions enhanced bacterial growth and enzyme production further. Optimal bacterial growth and enzyme production were obtained using ammonium sulphate and potassium phosphate together. Interpretations of the effects of added nutrients were complicated by the fact that steep nutrient gradients were formed in the timber as the nutrient salts diffused in. The results indicated that nutrient supplements did not alter the maximum size of the bacterial population but they did shorten the time taken to attain that maximum population.

Nitrogen and phosphate added to the sprinkling solution did stimulate pectinase production. Maximum concentrations of enzyme were found in squeezed sap from bolts sprinkled with nitrogen and phosphate together. The pH optimum of the pectinase assayed in squeezed sap was found to be between pH 8 and pH 9. The increase in sap pH from pH 5 to pH 7 implied that conditions inside logs sprinkled with phosphate buffer were more optimal for pectinase activity.

A time course examination of sprinkled wood with the scanning electron microscope demonstrated that the three pit

membrane types, simple pits, half-bordered pits and bordered pits were degraded at different rates. It seemed most likely that membrane accessibility to enzymatic degradation was responsible. Although enzymes were produced extracellularly, there was no evidence of extensive enzyme diffusion; pit degradation occurred only in close proximity to bacterial cells.

Degradation of pit membranes resulted in an increase in preservative uptake. As sprinkling time increased so did uptake, but the effect was non-linear. After an initial rise in preservative uptake in the first week of sprinkling, no further increase occurred for another four weeks. The uptakes actually fell slightly in some cases and that was attributed to the production of polysaccharide 'slimes' by the bacteria. Following the disappearance of the 'slime', preservative uptake increased further. The highest uptake in kerfed bolts occurred after seven weeks sprinkling with nitrogen and phosphate (3.6 times above that in non-sprinkled material). Similar trends were observed with non-kerfed wood but direct comparisons with the kerfed wood results were marred by end-seal failure.

Sprinkling also influenced the drying and shrinkage characteristics of Douglas fir sapwood. Measurements of radial and tangential unidirectional drying rates for the first four weeks of sprinkling were lower than the corresponding rates for non-sprinkled wood. The reduced rates were attributed to the presence of bacterial 'slimes' during that period. From five weeks onwards radial and tangential drying rates increased and were higher than non-sprinkled wood due to degraded pits and the subsequent

improvement in moisture flow.

The kerfs in sprinkled wood did not open up so wide as the kerfs in non-sprinkled wood when dried to the same equilibrium moisture content. The result suggested that tangential shrinkage in sprinkled wood was in some way reduced. The observation was confirmed from measurements of dimensional change in small cubes cut from sprinkled wood and non-sprinkled wood. Radial shrinkage remained unaltered. Possible mechanisms for the phenomenon were considered.

The exposed surfaces of sprinkled wood became stained during drying. The discolouration was confined to those areas colonised by bacteria and did not occur deeper than 1-2 mm from the surface of the timber. Migration of phenolic compounds normally present in the wood, but altered in some way by bacterial metabolism, was thought to be responsible. It was interesting to observe that stained areas inhibited colonisation of the wood by saprophytic fungi. It could not be ascertained whether the inhibition was due to the presence of bacteria or anti-fungal compounds in the stained zones or simply due to the staining reaction itself.

POTENTIAL AVENUES FOR FURTHER RESEARCH

The following suggestions provide interesting avenues for further research arising from these studies.

One of the most frustrating aspects of this study was the fact that the various microbiological aspects of the sprinkling process were not adequately investigated. The possibility of synergism between the component organisms of

the mixed inoculum was interesting, but not conclusively proved. The hypothesis merits further investigation both in-vitro and in-vivo. It is important to determine the individual contributions of the different organisms present in the mixed inoculum, particularly in the context of successional changes in the wood during the sprinkling process. It is possible that one or more of the organisms is superfluous. Any reduction in the number of organisms required for a mixed inoculum is an attractive proposition in terms of maintaining the inoculum in culture and guaranteeing its eventual capabilities.

Potentially, one of the most fruitful avenues is the application of the sprinkling process to other refractory species, for example Sitka spruce. Preliminary sprinkling trials with Sitka spruce were undertaken, but were not documented. The intial results were encouraging, suggesting that the sprinkling procedure used with Douglas fir was equally applicable to other species.

With regard to Douglas fir, the investigations described in this thesis go only part way towards understanding those factors influencing the sprinkling process. The properties of Douglas fir wood following sprinkling are largely unknown.

The preservative uptake results reported in this thesis were plagued by end-seal failure; it was therefore difficult to interpret the effects of different nutrient regimes and the effects of kerfing with a high degree of accuracy. End-seal failure could readily be resolved by using pole sized material (1.8m in length). Experiments with wood of larger dimensions would be more relevant to an industrial

situation. Large poles would also provide an opportunity to examine the influence of cyclic (on/off) sprinkling on the speed of bacterial migration and nutrient distribution in wood. The short length of the specimens used in this study may have been partly responsible for the inconclusive results obtained with cyclic sprinkling.

The Douglas fir roundwood placed in the sprinkling tanks was green, that is, it had a high moisture content. The wood eventually became totally saturated with sprinkling solution. It would be interesting to investigate whether partially dry wood would also become saturated. Wood available for commercial sprinkling operations is likely to be partially dry because of unavoidable delays involved with getting freshly felled logs from a forest to a suitable sprinkling plant. Permeability improvements could be less in partially dry wood because air embolisms might restrict the movement of bacteria and nutrients. The uptake of sprinkling solution probably plays an important role in the initial movement of bacteria and nutrient salts into the wood.

The inherently low moisture content of Douglas fir heartwood may render it resistant to bacterial attack during sprinkling. Of course, lignification during heartwood formation is also a major factor, but unless sufficient moisture is present in the wood it is unlikely that bacteria will survive and multiply. It would be useful to determine whether water saturated heartwood is more susceptible to bacterial attack than dry heartwood. The heartwood of a species such as Sitka spruce has a higher moisture content than Douglas fir and may therefore be more amenable to

permeability improvement.

Of more pressing interest is the behaviour of sprinkled, preservative treated wood in service. The shrinkage properties of sprinkled wood suggested that changes in the cell wall ultrastructure may have taken place along with some loss in strength. Strength losses in water-stored wood after prolonged storage in water have been noted (Liese 1984), but these have generally been considered minimal. Changes in the cell wall ultrastructure as a result of sprinkling should be examined more carefully with transmission electron microscopy.

It is possible that the absorption of ammonium salts by wood during sprinkling may make it more susceptible to decay in service. Conversely, it is also possible that the wood may be less susceptible to decay because the bacterial inoculum will have consumed all of the available soluble nutrients. Clearly, experiments to determine the decay susceptibility of sprinkled wood would prove useful.

The fate of the large bacterial population present in the wood, particularly the spore forming Bacillus spp., after preservative treatment poses an interesting question. The non-spore forming organisms will almost certainly be killed during the drying process and subsequent preservative treatment but the same may not be the case with the spore formers. It is likely that the preservative would inhibit significant bacterial spore germination and subsequent growth, but it would be of value to confirm that hypothesis.

The influence of potassium phosphate on the distribution of copper in preservative treated wood needs

further investigation. If, as the results from this study suggest, phosphate interferes with copper fixation, then although the permeability of the wood may be improved, preservative treatment with CCA may still be inadequate to provide decay resistance. It is also possible that even if adequate distribution of copper is obtained in preservative treated, sprinkled wood, the presence of phosphate may enhance leaching of copper in service. Different preservative formulations might be necessary for nutrient sprinkled wood or the nutrients could be removed prior to preservative treatment through leaching. However, a leaching treatment is unlikely to be economic.

It would be interesting to investigate how sprinkling affects the permeability of Douglas fir sawn timber. Certainly the exposed tangential faces of sawn timber provide a large surface area for bacterial entry. The relative rates of radial and tangential bacterial migration into sawn timber could be followed easily by sealing the radial or tangential faces prior to sprinkling.

ACKNOWLEDGEMENTS

First and foremost I would like to thank Drs. John Allen and John Walker who most ably supervised this project but gave me a free hand to follow up interesting observations along the way.

Special thanks must also go to Mrs Hilary Langer who provided valuable criticism of the experimental design and managed to extricate me from the horrors of statistical variation. Thanks also to Mrs Kay Card who endured many hours of scanning electron microscope operation and probably saw enough degraded bordered pits to last a lifetime.

Karl Schasching, Paul Fuller, Dave Clark, Rob Dalley, and Jeremy Young all deserve mention for not only keeping me sane but for providing indispensible technical help when required.

I am grateful to Dr. Bill Malcolm and his inumerable red pens for invaluable proof reading and a few lessons in basic grammar.

Many thanks to the post-graduate group at the School of Forestry both past and present for their competitive spirit, moral support and friendship. In particular, Dave, Francois, Helge, John, Mark, Peter, Roger, Ron and Tom.

The plentiful advice from Drs. A.L.J. Cole, J.R.L. Walker, B. Butterfield and L. Greenfield in the Botany Department was gratefully received.

Thanks to the personnel running the computer services at Canterbury, in particular Gillian Finnegan, for

unravelling the mysteries of computer plotting.

Thanks must also go to the New Zealand Forest Service for generously providing financial assistance for the three year project and to the Bank of New Zealand for the BNZ scholarship received in 1983.

Finally, without my wife Susan, who put up with a lot for four years, the whole thing would not have been possible.

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APPENDICES

APPENDIX M1

Determination of moisture content

Moisture contents were calculated from measurements of wet weight and oven dry weight using the following formula:

$$\text{Moisture content} = \frac{(\text{wet weight} - \text{oven dry weight})}{\text{oven dry weight}} \times \frac{100}{1}$$

APPENDIX M2

Determination of density

Specific densities were based on swollen (green) volume measurements using the following formula:

$$\text{Green density} = \frac{\text{green volume}}{\text{oven dry weight}}$$

APPENDIX M3

Sap pH measurements

Freshly cut samples were squeezed in a vice and the sap collected in a clean plastic bag. The pH was measured as quickly as possible with an ORION digital pH meter coupled to a standard ORION glass electrode.

APPENDIX M4

Bacterial counts

Previous authors, (Fogarty and Ward 1973, Dunleavy 1973, Macken and Pickaver 1979) have expressed bacterial counts as the number of colonies per millilitre of squeezed sap. However, comparisons can be difficult when dealing with wood of differing moisture contents. To avoid any problems, serial dilutions of macerated wood were plated out and the results were expressed as the number of bacterial colonies/gram of oven dry wood substance.

That technique involved removing a sample from a sprinkled bolt as aseptically as possible. The sample used was either an increment core removed with a sterile corer or a small wedge cut from a disc of wood, surface sterilised by

mild flaming. The wedge or core was divided into two portions. One half was weighed immediately then oven dried to calculate the percentage weight of wood substance in the sample. The other half was aseptically cut into small pieces, weighed, then homogenised in 100ml of sterile distilled water with a high speed blender (ULTRATURRAX). Homogenising for one minute was sufficient to produce a fibrous slurry of woody material. Serial dilutions were made into measured quantities of sterile distilled water. One millilitre of each 1:10 dilution was pipetted into a sterile petri dish and nutrient agar supplemented with 0.1% dextrose was poured over it. Three plates were made for each dilution and incubated at 25°C for five to seven days. After the incubation period discrete colonies on the three replicate plates were counted and the mean number of colonies obtained. The amount of wood substance (on an oven dry basis) in the original sample was estimated by multiplying the weight of the sample actually homogenised by the percentage weight of wood substance in the sample (determined from the piece adjacent). Bacterial counts were divided by this value to give the number of bacteria per gram of oven dry weight of wood.

APPENDIX M5

Spectrophotometric assay of pectinase activity.

Crude enzyme extracts (squeezed sap) were centrifuged at 8000 X g for 1 minute to remove bacterial cells. The cell free supernatant was dialysed against two changes of 0.05M Tris buffer at 4 C for approximately 24 h. Dialysed extracts were kept in an ice bath until used.

The enzyme assay was carried out in a temperature controlled room at 25 °C (a temperature controlled room cuvette was not available). The temperature of all glassware, cuvettes, reagents and the spectrophotometer were allowed to equilibriate for 24h. A 0.05% solution of polygalacturonic acid (SIGMA) substrate was prepared in 0.05M Tris buffer pH 8 containing 0.001M CaCl . 2.9ml of the substrate were mixed with 0.1ml of the cell free enzyme solution. The increase in absorbance at 235nm over time was measured using a Shimadzu double beam spectrophotometer. The substrate without enzyme served as a blank.

APPENDIX M6

Detection of pectinase activity on solid agar

Pectinase activity was detected by flooding cultures grown on the following medium for seven days with hexadecyltrimethylammonium bromide.

$(NH_4)_2SO_4$	2g
KH_2PO_4	4g
Na_2HPO_4	6g
$FeSO_4$	0.001g
$MgSO_4$	0.2g
$CaCl_2$	0.001g
$MnSO_4$	trace
$ZnSO_4$	trace
pectin	5g
agar	15g
yeast extract	1g
distilled water	1l

Zones of pectolysis appeared as clear zones.

APPENDIX M7

Biochemical tests used for bacterial identification

All bacteria were incubated at 30 °C.

M 7.1 Gram staining

Air dry smears of 24h old cultures were made on ethanol cleaned slides. After heat fixing, the smears were stained with crystal violet for two minutes and then rinsed with grams iodine for 1 minute. Stained smears were washed in 95% ethanol for approximately 1 minute until no further colour change occurred and then counterstained with carbol fuchsin. Stained cultures were examined with a light microscope.

M 7.2 Fermentative growth

Fermentative growth was assessed using Hugh and Liefsons medium (Hugh R. and Liefson E. 1953, Journal of Bacteriology 66, 24). The following basal medium was prepared:

Peptone	0.2g
NaCl	0.5g
K_2HPO_4	0.03g
Agar	0.3g
Bromothymol blue	0.3ml of 1% aqueous solution
Distilled water	100ml

Final pH 7.1

A ten percent solution of glucose was autoclaved separately and added to the previously sterilised basal medium. The complete medium was dispensed into sterile McCartney bottles. Two tubes of medium were stab inoculated with the bacterium under test, one tube was covered with a layer of sterile paraffin oil to exclude air. After 7 days incubation the tubes were examined for evidence of growth. Fermentative organisms produced acid in both tubes (revealed by colour change from blue to yellow). Oxidative organisms produced acid only in the tube without oil.

M 7.3 Motility

A standard hanging drop preparation was made from a 24h old culture and examined under oil for movement.

M 7.4 Catalase activity

A loopful of bacterial culture was emulsified in a drop of hydrogen peroxide on a slide. Effervescence in the emulsion was taken to be a positive sign of catalase activity.

M 7.5 Acid fastness

The Ziehl-Neelsen method was used to determine acid fastness. Smears were heated with carbol fuchsin without boiling for 5 minutes, washed in distilled water then decolourised with 20% sulphuric acid. After washing once more the smears were counterstained with methylene blue and examined. Smears retaining carbol fuchsin stain were acid fast.

M 7.6 Anaerobic growth

Inoculated nutrient agar plates were placed into a BBL anaerobic jar containing a BBL GAS PAK hydrogen generator. After incubation at 30°C for 7 days, plates were removed and examined for evidence of bacterial growth.

M 7.7 Heat survival

Three day nutrient broth cultures of each bacterium were placed into an 80 °C water bath for 10-15 minutes. Cultures were removed, cooled and subcultured onto solid nutrient agar media. Evidence of growth after 7 days incubation at 30°C was interpreted as survival of the heat treatment.

M 7.8 Starch utilisation

One percent soluble starch was added to solid nutrient agar media forming 'starch' agar. Starch agar plates were streak inoculated with a culture and incubated. After 7 days plates were flooded with Gram's iodine. Unhydrolysed starch forms a blue colour, hydrolysed starch remains clear.

M 7.9 Vosges proskauer test

O'Meara's modification of the standard test was used. Cultures were grown in a glucose phosphate broth for 5 days and then a small amount of creatine was added followed by 5ml of 40% NaOH. After vigorous shaking for 60 seconds the mixture was left to stand. A positive test was indicated if a pink colour developed within 30 minutes.

M 7.10 Acid and gas production from sugars

Cultures were stab inoculated into the following medium:

peptone	5g
yeast extract	3g
NaCl	5g
agar	3g
bromocresol purple	0.008g
Glucose	10g
distilled water	1000ml
pH 7	

Glucose was added to the medium after sterilisation. Alternative sugars were substituted for glucose at the same concentration of 10g/l. Gas production was obvious by the appearance of bubbles in the medium, acid production by a change in the dye colour from purple to yellow.

APPENDIX M8

Measurement of culture pH, reducing sugar level and pectinase activity in flasks containing wood cubes and nutrient media.

Culture pH was measured directly with an ORION glass electrode. Reducing suagar concentration was measured using the 3,5 -dinitrosalicylic acid (DNSA) reagent as follows:

2.5g of DNSA was dissolved in 50ml 2M NaOH solution and 125ml water. 75g of Na-K-tartrate was then added and when dissolved the solution was made up to 250ml with water. One part of the sample (culture fluid) was added to two parts of DNSA reagent and heated in a boiling water bath for at least 4 minutes. After cooling to room temperature the absorbance at 570nm relative to a blank made up of DNSA reagent and water was recorded (Lindsay H., 1973, Potato Research 16(2), 176-179).

Pectinase activity in culture fluid was assessed through an estimation of the concentration of C-4,5 unsaturated oligouronide pectin breakdown products. A brief description of the periodate/thiobarbituric acid test used for the analysis is as follows:

1ml of culture fluid was added to 1.25ml of 0.025N periodic acid in 0.125N sulphuric acid. After 20 minutes at room temperature 2.5ml of sodium arsenite in 0.5N HCl were added with shaking and the solution was allowed to stand for

two minutes. 10ml of 0.3% thiobarbituric acid (pH 2) was added and the mixture was heated in a boiling water bath for 10 minutes. A salmon pink colour developed in the presence of pectin breakdown products. After cooling the optical density of the solution was measured at 548nm and compared to that of a distilled water blank (Fuchs A., 1965, Antonie van Leeuwenhoek 31 323-340).

APPENDIX M9

Measurement of catalase activity.

The catalase technique of Line (1983) was employed to estimate the number of bacteria present in Douglas fir cubes at different times after inoculation. The technique was as follows:

At each sampling time one cube was removed from each flask aseptically and washed in sterile distilled water. A sterilised coping saw was then used to reduce half of each cube to moist saw dust. 0.5g of saw dust was added to 60ml of partially de-aerated 0.05M phosphate buffer pH 7. The sawdust buffer mixture was gently stirred with a magnetic stirrer and an ORION oxygen electrode, coupled to an ORION digital ion analyser, was partially immersed in the solution. Using such a set up it was possible to obtain a reading of the dissolved oxygen concentration in parts per million. The dissolved oxygen level was allowed to stabilise for several minutes and then sufficient hydrogen peroxide was added to give a final concentration of 0.01%. The rate of oxygen evolution, due to catalase activity was followed for 60 seconds using a chart recorder. Rates were adjusted by subtracting the low rate of oxygenation which occurred with buffer and sawdust alone. A standard curve of number of bacteria versus ppm oxygen evolved per minute was used to convert the oxygen evolution readings to numbers of bacteria.

APPENDIX R1

Previous history of the wood used for sprinkling trials.

All of the Douglas fir poles used in this study were cut from trees felled at Ashley forest, Canterbury between November 1982 and May 1984. Most timber was extracted from Compartment 38, Stand 1 originally planted in 1958 with Ashley 'strain' Douglas fir. Some confusion exists over the origin of the seed source and its relationship to Coastal or Interior, American Douglas fir. Therefore no prior knowledge of the timber's treatability to preservative was known.

It was generally possible to cut at least four 1.8m poles from each tree felled. The butt pole was usually too large to fit into the sprinkling tanks and was therefore discarded. This left three remaining useful poles which were labelled with a tree number and either 'A', 'B' or 'C' depending on the pole's position relative to the ground. Thus pole '8C' was the top pole from tree 8 and so on.

To reduce the amount of drying after felling, poles were transported back to the School of Forestry immediately. The cut ends of the poles were sealed with a thick coat of 'FLINTCOTE', placed in polythene sleeving and kept at 4 C until needed. Storage in this manner kept the poles in as green a state as was possible for many months.

APPENDIX R2

Details of poles used in the kerfed sprinkling trial.

Pole 11C - Mean S.E.R. 62mm
Mean L.E.R. 75mm
Mean sapwood density 390 kg/m3
Bolts 4918,4919,4917 and 4916 sprinkled for 1 week

Pole 8C - Mean S.E.R. 70mm
Mean L.E.R. 78mm
Mean sapwood density 340 kg/m3
Bolts 4900,4901,4902 and 4903 sprinkled for 2 weeks

Pole 9C - Mean S.E.R. 61mm
Mean L.E.R. 71mm
Mean sapwood density 380 kg/m3
Bolts 4909,4911,4908 and 4910 sprinkled for 3 weeks

Pole 10C Mean S.E.R. 55mm
Mean L.E.R. 68mm
Mean sapwood density 410 kg/m3
Bolts 4912,4914,4913 and 4915 sprinkled for 4 weeks

Pole 3C Mean S.E.R. 58mm
Mean L.E.R. 76mm
Mean sapwood density 376 kg/m3
Bolts 4923,4920,4921 and 4922 sprinkled for 5 weeks

Pole 12C Mean S.E.R. 60mm
Mean L.E.R. 68mm
Mean sapwood density 380 kg/m3
Bolts 4899,4898,4897 and 4896 sprinkled for 7 weeks

Pole 4C Mean S.E.R. 58mm
Mean L.E.R. 68mm
Mean sapwood density 420 kg/m3
Bolts 4904,4905,4906 and 4907 not sprinkled

Pole 6C Mean S.E.R. 58mm
Mean L.E.R. 70mm
Mean sapwood density 380 kg/m3
Bolts 4952 and 4951 not sprinkled

Pole 7C Mean S.E.R 55mm
Mean L.E.R. 65mm
Mean sapwood density 380 kg/m3
Bolts 3001 and 3002 not sprinkled

Details of the poles used in the non-kerfed sprinkling trials.

Pole 8B
Mean S.E.R 65mm
Mean L.E.R 73mm
Mean sapwood density 420kg/m3
Bolts 4924,4925,4926,4927 sprinkled for 8 weeks

Pole 9B
Mean S.E.R 75mm
Mean L.E.R 80mm
Mean sapwood density 400kg/m3
Bolts 4928,4929,4930,4931 sprinkled for 6 weeks

Pole 6B
Mean S.E.R 65mm
Mean L.E.R 77mm
Mean sapwood density 430kg/m3
Bolts 4932,4933,4934,4935 sprinkled for 4 weeks

Pole 2B
Mean S.E.R 74mm
Mean L.E.R 82mm
Mean sapwood density 450kg/m3
Bolts 4940,4941,4942,4943 sprinkled for 2 weeks

Pole 12B
Mean S.E.R 77mm
Mean L.E.R 83mm
Mean sapwood density 390kg/m3
Bolts 4944,4945,4946,4947 sprinkled for 3 weeks

Pole 7B
Mean S.E.R 70mm
Mean L.E.R 75mm
Mean sapwood density 410kg/m3
Bolts 4948,4949,4950,4951 not sprinkled

APPENDIX R 3 Moisture content and density data relating to the sprinkling of kerfed Douglas fir.

ID	SPRINKLING TIME (WEEKS)	TREATMENT	MEAN DENSITY (Kg/m3)	WEIGHT BEFORE SPRINKLING (g)	WEIGHT AFTER SPRINKLING (g)	INITIAL MEAN PERCENT SATURATION	FINAL MEAN PERCENT SATURATION	INITIAL HEARTWOOD M.C.	FINAL HEARTWOOD M.C.
4918	1	1	390	6184.4	6536.1	83.2	82.5	47.4	47.3
4919	1	2	390	5166.8	5443.3	83.2	93.2	47.4	57.0
4917	1	3	390	5911.8	6278.6	83.2	87.7	47.4	55.9
4916	1	4	390	6003.8	6409.5	83.2	91.3	47.4	59.4
4902	2	1	340	5494.7	5774.3	83.2	85.7	49.7	51.5
4901	2	2	340	6283.4	6637.5	83.2	83.5	49.7	64.9
4900	2	3	340	7191.8	7781.8	83.2	82.7	49.7	52.1
4903	2	4	340	6463.2	6896.3	83.2	83.9	49.7	47.2
4909	3	1	380	6037.8	6620.9	83.2	85.1	46.3	57.8
4911	3	2	380	5990.5	6540.5	83.2	85.6	46.3	53.8
4908	3	3	380	5183.9	5665.9	83.2	91.6	46.3	51.3
4910	3	4	380	6426.0	6993.1	83.2	81.2	46.3	55.5
4912	4	1	410	5222.1	5633.6	83.2	92.7	52.7	74.8
4914	4	2	410	4334.6	4667.7	83.2	97.1	52.7	84.4
4913	4	3	410	5864.6	6357.7	83.2	93.6	52.7	67.3
4915	4	4	410	5669.8	6120.6	83.2	94.4	52.7	103.7
4923	5	1	376	6725.6	7214.1	83.2	89.9	51.3	58.9
4920	5	2	376	5981.9	6360.5	83.2	92.2	51.3	58.5
4921	5	3	376	6491.9	6930.4	83.2	96.7	51.3	51.9
4922	5	4	376	5645.7	6103.7	83.2	92.9	51.3	56.9
4899	7	1	380	5122.6	5692.8	83.2	97.0	51.0	53.4
4898	7	2	380	5893.9	6469.3	83.2	97.1	51.0	58.3
4897	7	3	380	4520.8	4982.5	83.2	98.8	51.0	54.9
4896	7	4	380	5931.1	6500.9	83.2	97.2	51.0	54.2

APPENDIX R 4 Basic data relating to the moisture content and density of non-kerfed, sprinkled Douglas fir before and after sprinkling.

ID	SPRINKLING TIME (WEEKS)	TREATMENT	MEAN DENSITY (kg/m3)	WEIGHT BEFORE SPRINKLING (g)	WEIGHT AFTER SPRINKLING (g)	INITIAL MEAN PERCENT SATURATION	FINAL MEAN PERCENT SATURATION	INITIAL HEARTWOOD M.C.	FINAL HEARTWOOD M.C.
4940	2	1	450	6399.2	6959.7	80.2	84.6	48.06	51.30
4941	2	2	450	6901.9	7463.3	80.2	79.0	48.06	48.17
4943	2	3	450	6522.6	7031.8	80.2	86.6	48.06	51.28
4942	2	4	450	6388.0	6932.7	80.2	86.6	48.06	43.32
4945	3	1	390	7748.3	8395.3	84.6	88.9	43.47	45.06
4944	3	2	390	7631.6	8233.0	84.6	94.3	43.47	43.81
4946	3	3	390	6265.9	6611.5	84.6	96.6	43.47	47.61
4947	3	4	390	7101.9	7700.6	84.6	90.1	43.47	42.98
4932	4	1	430	7821.9	8579.4	85.3	88.9	44.34	47.33
4933	4	2	430	7342.7	8109.5	85.3	92.9	44.34	55.02
4934	4	3	430	6802.8	7458.4	85.3	97.1	44.34	52.78
4935	4	4	430	7720.7	8527.8	85.3	90.7	44.34	47.37
4928	6	1	400	6318.0	6868.0	83.7	94.5	49.34	49.89
4931	6	2	400	6508.8	7105.5	83.7	94.7	49.34	49.66
4930	6	3	400	5620.0	6001.8	83.7	97.5	49.34	50.64
4929	6	4	400	6479.4	7073.0	83.7	97.8	49.34	49.29
4924	8	1	420	5768.0	6294.4	81.3	93.9	47.25	51.58
4927	8	2	420	6214.2	6816.6	81.3	99.3	47.25	53.15
4926	8	3	420	6144.5	6547.6	81.3	89.0	47.25	49.17
4925	8	4	420	6207.9	6853.1	81.3	95.5	47.25	51.59

M.C = MOISTURE CONTENT

APPENDIX R 5 DATA RELATING TO SEM-EDAX ANALYSIS FOR POTASSIUM,PHOSPHORUS AND SULPHUR IN KERFED,SPRINKLED DOUGLAS FIR

BOLT ID	SPRINKLING TIME (WEEKS)	POSITION IN DISC	ELEMENT (MEAN COUNTS PER SECOND) POTASSIUM	PHOSPHORUS	SULPHUR
4900	2	A1	10.95	____	____
		S.E.	3.95	____	____
		A2	____	____	____
		S.E.	____	____	____
		A3	____	____	____
		S.E.	____	____	____
		X1	____	____	____
		S.E.	____	____	____
4908	3	A1	107.72	36.51	22.89
		S.E.	7.67	3.46	2.63
		A2	19.08	0.74	1.27
		S.E.	2.83	0.74	1.27
		A3	6.22	____	____
		S.E.	1.49	____	____
		X1	5.35	____	____
		S.E.	1.51	____	____
4913	5	A1	191.25	61.11	38.00
		S.E.	13.24	11.30	5.92
		A2	175.62	39.13	28.01
		S.E.	9.21	3.51	2.99
		A3	26.89	1.09	2.35
		S.E.	13.35	1.09	2.35
		X1	3.35	____	____
		S.E.	1.51	____	____
4897	7	A1	285.93	93.58	53.43
		S.E.	28.16	13.63	8.06
		A2	151.70	40.16	23.61
		S.E.	19.90	8.78	4.58
		A3	102.32	26.00	18.21
		S.E.	15.00	5.91	3.22
		X1	21.21	1.08	1.49
		S.E.	5.43	1.08	1.49

____ INDICATES THAT THE AMOUNT OF ELEMENT PRESENT WAS TOO LOW FOR DETECTION

S.E. = STANDARD ERROR OF THE MEAN

APPENDIX R 6 pH DATA FOR SQUEEZED SAP SAMPLED FROM KERFED DOUGLAS FIR BOLTS AT DIFFERENT SPRINKLING TIMES

SPRINKLING TIME (WEEKS)	TREATMENT	POSITION IN DISC 0	1A	2A	3A	4A	1B	2B	3B	4B
0	(CONTROL)	5.1	4.8	4.8	4.8	5.1	___	___	___	___
4	1	6.3	6.1	___	5.2	___	5.1	___	6.1	___
	2	5.1	4.7	___	4.8	___	4.8	___	4.7	___
	3	6.7	6.3	___	4.8	___	4.9	___	6.1	___
	4	5.7	4.8	___	4.7	___	4.8	___	4.9	___
5	1	7.1	6.7	5.9	5.9	5.7	5.1	5.4	5.8	6.8
	2	5.4	4.6	___	4.7	___	4.7	___	4.7	___
	3	6.4	6.2	4.9	4.8	4.7	5.0	5.4	5.4	6.3
	4	6.4	___	5.0	___	4.9	___	4.8	___	5.2
7	1	6.8	6.7	5.9	5.5	5.0	5.1	5.3	5.9	6.8
	2	5.3	4.8	___	4.6	___	4.7	___	4.6	___
	3	6.2	6.3	5.9	5.0	4.8	4.9	5.3	5.5	6.1
	4	6.2	4.8	___	4.5	___	4.6	___	4.8	___

(___) INDICATES THAT NO MEASUREMENTS WERE TAKEN

APPENDIX R 7 BACTERIAL NUMBERS FROM PLATE COUNTS OF MACERATED WOOD TISSUE REMOVED FROM KERFED, SPRINKLED DOUGLAS FIR AT DIFFERENT SPRINKLING TIMES.

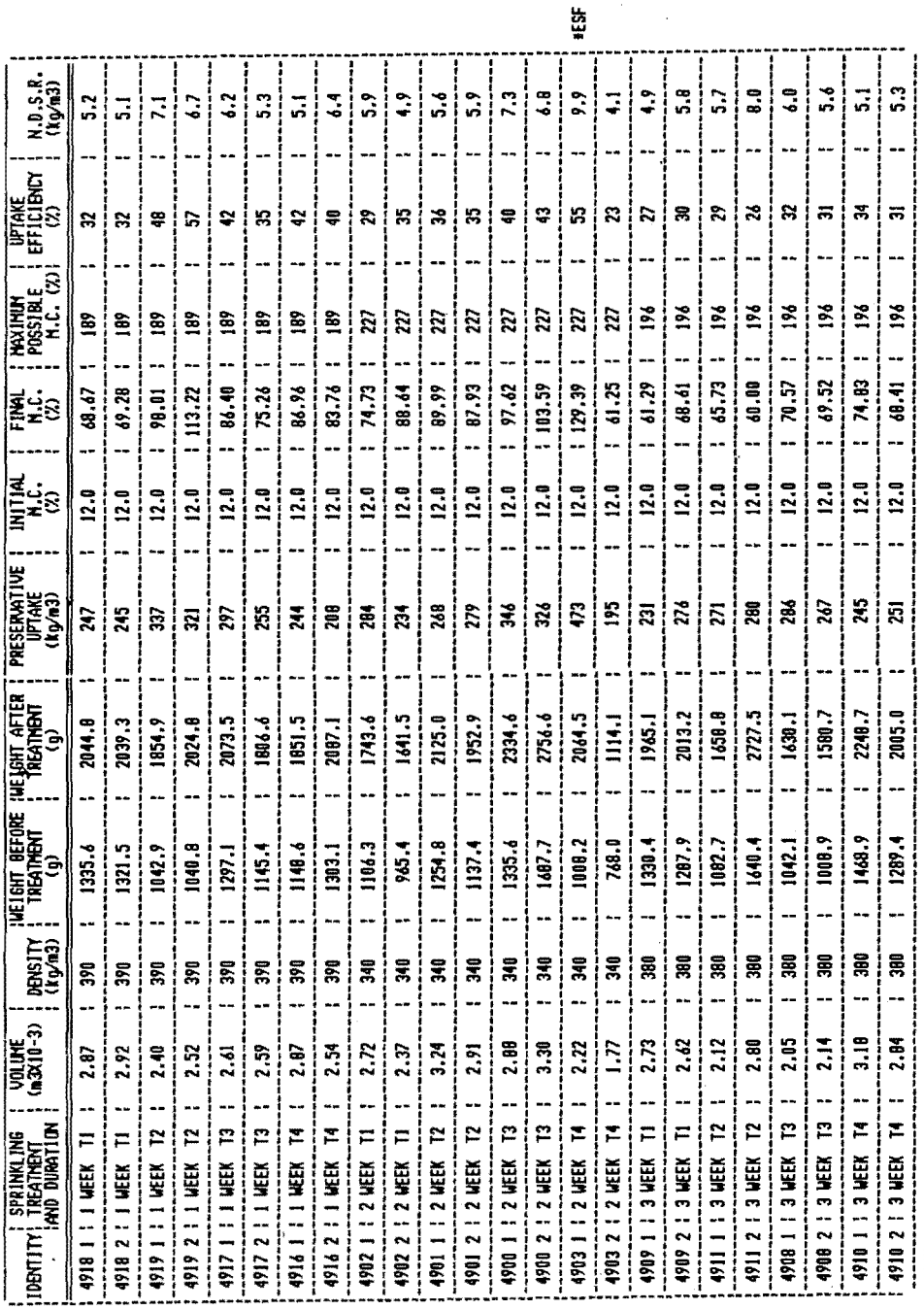
NUMBERS REPRESENT RAW COUNTS X 1 MILLION PER GRAM OF WOOD TISSUE ON AN OVEN DRY BASIS.

TREATMENT	SAMPLE	WEEK 1	WEEK2	WEEK3	WEEK4	WEEK5	WEEK7
1 BUFFER	X	355	850	161	1360	84.4	820
	X1	____	____	4.8	2.5	3.4	190
	A1C1	8.2	47	86.1	189	1050	4850
	A2C2	5.6	5.4	183	27.5	240	847
	A3C3	5.4	11	11.7	32.7	249	163
2 NITROGEN	X	640	819	103	859	424	380
	X1	____	____	2.2	1.5	6.7	74.7
	A1C1	4.5	24	87.4	107	359	100
	A2C2	1.9	8.9	93.7	49.2	22.6	48.9
	A3C3	4.8	18	6.95	8.7	22.0	22.7
3 NITROGEN + BUFFER	X	610	454	909	2010	169	1100
	X1	____	____	1.2	10	20.4	247
	A1C1	12	19	496	2350	2130	2545
	A2C2	0.87	24	21.9	743	1260	8945
	A3C3	6.8	39	8.9	136	162	770
4 WATER	X	71.4	32	170	1360	1110	519
	X1	____	____	2.7	0.9	9.7	0.6
	A1C1	2.7	23	8.1	100	21.9	65
	A2C2	0.8	9.4	2.0	9.9	8.4	101
	A3C3	0.87	11	2.9	16.4	14.0	15.5

(____) INDICATES THAT NO SAMPLES WERE TAKEN

APPENDIX R 8 Preservative treatment raw data for sprinkled kerfed Douglas fir.

Part A



IDENTITY	SPRINKLING TREATMENT AND DURATION	VOLUME (m3X10-3)	DENSITY (kg/m3)	WEIGHT BEFORE TREATMENT (g)	WEIGHT AFTER TREATMENT (g)	PRESERVATIVE UPTAKE (kg/m3)	INITIAL M.C. (%)	FINAL M.C. (%)	MAXIMUM POSSIBLE M.C. (%)	UPTAKE EFFICIENCY (%)	N.D.S.R. (kg/m3)
4918 1 1 1	WEEK T1	2.87	390	1335.6	2044.8	247	12.0	68.67	189	32	5.2
4918 2 1 1	WEEK T1	2.92	390	1321.5	2039.3	245	12.0	69.28	189	32	5.1
4919 1 1 1	WEEK T2	2.40	390	1042.9	1854.9	337	12.0	98.01	189	48	7.1
4919 2 1 1	WEEK T2	2.52	390	1040.8	2024.8	321	12.0	113.22	189	57	6.7
4917 1 1 1	WEEK T3	2.61	390	1297.1	2073.5	297	12.0	86.40	189	42	6.2
4917 2 1 1	WEEK T3	2.59	390	1145.4	1806.6	255	12.0	75.26	189	35	5.3
4916 1 1 1	WEEK T4	2.87	390	1148.6	1851.5	244	12.0	86.96	189	42	5.1
4916 2 1 1	WEEK T4	2.54	390	1303.1	2087.1	208	12.0	83.76	189	40	6.4
4902 1 1 2	WEEK T1	2.72	340	1106.3	1743.6	284	12.0	74.73	227	29	5.9
4902 2 1 2	WEEK T1	2.37	340	965.4	1641.5	234	12.0	88.64	227	35	4.9
4901 1 1 2	WEEK T2	3.24	340	1254.8	2125.0	268	12.0	89.99	227	36	5.6
4901 2 1 2	WEEK T2	2.91	340	1137.4	1952.9	279	12.0	87.93	227	35	5.9
4900 1 1 2	WEEK T3	2.88	340	1335.6	2334.6	346	12.0	97.62	227	40	7.3
4900 2 1 2	WEEK T3	3.30	340	1687.7	2756.6	326	12.0	103.59	227	43	6.8
4903 1 1 2	WEEK T4	2.22	340	1008.2	2064.5	473	12.0	129.39	227	55	9.9
4903 2 1 2	WEEK T4	1.77	340	768.0	1114.1	195	12.0	61.25	227	23	4.1
4909 1 1 3	WEEK T1	2.73	380	1330.4	1965.1	231	12.0	61.29	196	27	4.9
4909 2 1 3	WEEK T1	2.62	380	1287.9	2013.2	276	12.0	68.61	196	30	5.8
4911 1 1 3	WEEK T2	2.12	380	1082.7	1658.8	271	12.0	65.73	196	29	5.7
4911 2 1 3	WEEK T2	2.80	380	1640.4	2727.5	280	12.0	60.80	196	26	8.0
4908 1 1 3	WEEK T3	2.05	380	1042.1	1630.1	286	12.0	70.57	196	32	6.0
4908 2 1 3	WEEK T3	2.14	380	1008.9	1580.7	267	12.0	69.52	196	31	5.6
4910 1 1 3	WEEK T4	3.18	380	1468.9	2240.7	245	12.0	74.83	196	34	5.1
4910 2 1 3	WEEK T4	2.84	380	1289.4	2005.0	251	12.0	68.41	196	31	5.3

#ESF

ABBREVIATIONS: N.D.S.R. NET DRY SALT RETENTION BASED ON 2.1% TANALITH NCA
M.C. MOISTURE CONTENT
ESF END-SEAL FAILURE

IDENTITY	SPRINKLING TREATMENT AND DURATION	VOLUME (m3X10-3)	DENSITY (kg/m3)	WEIGHT BEFORE TREATMENT (g)	WEIGHT AFTER TREATMENT (g)	PRESERVATIVE UPTAKE (kg/m3)	INITIAL M.C. (%)	FINAL M.C. (%)	MAXIMUM POSSIBLE M.C. (%)	UPTAKE EFFICIENCY (%)	N.D.S.R. (kg/m3)
4912 1	4 WEEK T1	1.57	410	897.0	1429.4	339	12.0	86.08	177	45	7.1
4912 2	4 WEEK T1	2.09	410	1133.0	1765.6	302	12.0	68.50	177	34	6.3
4914 1	4 WEEK T2	1.60	410	849.5	1409.3	349	12.0	76.63	177	39	7.3
4914 2	4 WEEK T2	1.52	410	900.6	1402.8	330	12.0	72.64	177	37	6.9
4913 1	4 WEEK T3	2.36	410	1185.2	1739.1	234	12.0	58.80	177	28	4.9
4913 2	4 WEEK T3	3.35	410	1114.0	1700.4	175	12.0	65.88	177	33	3.7
4915 1	4 WEEK T4	2.43	410	1231.3	1824.2	243	12.0	61.55	177	30	5.1
4915 2	4 WEEK T4	1.72	410	1028.7	1558.6	308	12.0	65.07	177	32	6.5
4923 1	5 WEEK T1	3.09	376	1423.2	2283.3	278	12.0	77.14	199	35	5.8
4923 2	5 WEEK T1	3.05	376	1438.6	2307.8	285	12.0	75.19	199	34	5.8
4920 1	5 WEEK T2	2.56	376	1213.2	2254.1	406	12.0	103.77	199	49	8.5
4920 2	5 WEEK T2	2.40	376	1106.5	2038.5	388	12.0	100.12	199	47	8.1
4921 1	5 WEEK T3	2.29	376	1050.7	1989.6	410	12.0	82.63	199	38	8.6
4921 2	5 WEEK T3	3.09	376	1530.8	2950.9	459	12.0	106.02	199	50	9.6
4922 1	5 WEEK T4	1.72	376	1658.0	2281.5	362	12.0	100.07	199	47	7.6
4922 2	5 WEEK T4	1.63	376	753.4	1256.1	308	12.0	81.16	199	37	6.5
4899 1	7 WEEK T1	2.26	380	1031.8	2094.8	470	12.0	125.45	196	62	9.9
4899 2	7 WEEK T1	1.95	380	1075.6	1965.1	456	12.0	115.50	196	56	9.6
4898 1	7 WEEK T2	2.10	380	1060.0	2071.3	481	12.0	108.82	196	53	10.6
4898 2	7 WEEK T2	2.80	380	1241.8	2561.6	471	12.0	121.48	196	59	9.9
4897 1	7 WEEK T3	1.92	380	936.9	1953.7	529	12.0	137.29	196	68	11.1
4897 2	7 WEEK T3	1.53	380	970.4	1793.2	537	12.0	125.53	196	62	11.3
4896 1	7 WEEK T4	2.07	380	1020.2	1809.4	381	12.0	91.04	196	43	8.0
4896 2	7 WEEK T4	2.91	380	1418.8	2449.2	354	12.0	101.77	196	49	7.4

ABBREVIATIONS: N.D.S.R. NET DRY SALT RETENTION USING 2.1% TANALITH NCA
M.C. MOISTURE CONTENT
ESF END-SEAL FAILURE

IDENTITY	SPRINKLING TREATMENT AND DURATION	VOLUME (m3X10-3)	DENSITY (kg/m3)	WEIGHT BEFORE TREATMENT (g)	WEIGHT AFTER TREATMENT (g)	PRESERVATIVE UPTAKE (kg/m3)	INITIAL M.C. (%)	FINAL M.C. (%)	MAXIMUM POSSIBLE M.C.(%)	UPTAKE EFFICIENCY (%)	N.D.S.R (kg/m3)
4904 1	CONTROL	2.68	410	1419.8	1645.9	84	12.0	29.40	177	10	1.8
4904 2	CONTROL	2.40	410	1335.5	1701.5	152	12.0	35.23	177	14	3.2
4906 1	CONTROL	2.46	410	1267.5	1565.2	142	12.0	32.70	177	13	3.0
4906 2	CONTROL	3.00	410	1639.1	2028.1	130	12.0	34.54	177	14	2.7
4907 1	CONTROL	3.79	410	1895.7	2253.7	109	12.0	34.71	177	14	2.3
4907 2	CONTROL	2.55	410	1567.3	1982.5	162	12.0	38.75	177	16	3.4
4905 1	CONTROL	2.33	410	1183.3	1588.0	174	12.0	39.99	177	17	3.6
4905 2	CONTROL	2.10	410	1127.0	1480.9	168	12.0	43.78	177	19	3.5
3001 1	CONTROL	1.66	380	844.3	1160.1	190	12.0	48.94	196	20	4.0
3001 2	CONTROL	1.84	380	861.0	1180.1	173	12.0	49.78	196	20	3.6
3002 2	CONTROL	2.14	380	1180.1	1494.1	146	12.0	44.53	196	17	3.1
3002 1	CONTROL	3.65	380	1728.5	2285.0	152	12.0	40.25	196	15	3.2

ABBREVIATIONS: N.D.S.R. NET DRY SALT RETENTION USING 2.1% TANALITH NCA
M.C. MOISTURE CONTENT
ESF END-SEAL FAILURE

APPENDIX R 9 RADIAL AND TANGENTIAL PENETRATION OF CCA PRESERVATIVE INTO KERFED, SPRINKLED DOUGLAS FIR

SPRINKLING TIME (WEEKS)	TREATMENT	RADIAL PENETRATION (mm)	TANGENTIAL PENETRATION (% OF CIRCUMFERENCE)
1	1	13.0	17.5
	2	17.5	21.5
	3	12.0	20.8
	4	11.5	21.9
2	1	12.2	6.1
	2	15.5	12.6
	3	16.0	17.9
	4	10.0	4.2
3	1	11.5	15.4
	2	7.5	15.0
	3	11.0	16.1
	4	9.5	12.4
4	1	8.0	23.0
	2	9.0	23.0
	3	10.0	14.8
	4	9.0	17.0
5	1	10.0	17.2
	2	11.0	36.0
	3	9.0	24.8
	4	12.0	21.5
7	1	17.0	57.0
	2	14.0	85.4
	3	___	100.0
	4	13.0	32.0

APPENDIX R10 BACTERIAL NUMBERS IN SPRINKLED, NON-KERFED DOUGLAS FIR AT DIFFERENT TIMES. NUMBERS WERE OBTAINED FROM PLATE COUNTS OF MACERATED WOOD TISSUE. THE FIGURES REPRESENT NUMBERS X 1 MILLION PER GRAM OF WOOD TISSUE ON AN OVEN DRY BASIS.

TREATMENT	SAMPLE LOCATION	SPRINKLING TIME (WEEKS) 2	3	4	6	8.5
BUFFER	OUTER SAP	99.2	328	2750	8800	10000
	INNER SAP	0.83	7.5	0.65	2.56	4.9
NITROGEN	OUTER SAP	86	1910	1080	588	1290
	INNER SAP	2.7	2.1	1.9	3.9	1.7
BUFFER + NITROGEN	OUTER SAP	317	349	450	3630	2490
	INNER SAP	1.6	1.6	1.7	40	33
WATER	OUTER SAP	90.4	500	263	1317	320
	INNER SAP	1.7	3.6	1.6	0.8	3.7

APPENDIX R11 DATA RELATING TO SEM-EDAX ANALYSIS OF POTASSIUM, PHOSPHORUS AND SULPHUR IN NON-KERFED, SPRINKLED DOUGLAS FIR

BOLT ID	SPRINKLING TIME (WEEKS)	POSITION	ELEMENT (MEAN COUNTS PER SECOND) POTASSIUM	PHOSPHORUS	SULPHUR
4943	2	OUTER SAP	59.51	18.18	9.34
		S.E.	18.46	6.70	4.39
		INNER SAP	1.08	_____	_____
		S.E.	1.08	_____	_____
4946	3	OUTER SAP	88.53	21.42	9.77
		S.E.	9.01	3.57	2.54
		INNER SAP	2.44	_____	_____
		S.E.	1.51	_____	_____
4934	4	OUTER SAP	200.56	62.36	32.57
		S.E.	10.29	1.89	3.14
		INNER SAP	3.71	____	____
		S.E.	1.65	____	____
4930	6	OUTER SAP	220.00	57.08	29.28
		S.E.	20.40	5.95	2.66
		INNER SAP	58.76	8.87	5.34
		S.E.	10.98	2.90	1.44
4926	8.5	OUTER SAP	112.48	28.61	14.48
		S.E.	14.23	6.24	2.07
		INNER SAP	69.25	12.94	10.97
		S.E.	5.60	1.74	1.70

(_____) INDICATES THAT THE AMOUNT OF ELEMENT PRESENT IN THE SAMPLE WAS TOO LOW FOR DETECTION

(S.E.) = STANDARD ERROR OF THE MEAN

APPENDIX R12 pH MEASUREMENTS IN SQUEEZED SAP AND SPRINKLING TANKS FOR NON-KERFED DOUGLAS FIR

TREATMENT	LOCATION	SPRINKLING TIME (WEEKS) 0	2	3	4	6	8.5
1	TANK	7.4	7.4	7.4	7.4	7.5	7.5
	INNER SAP	5.0	5.2	5.3	5.4	5.0	5.0
	OUTER SAP	5.7	6.8	6.9	6.8	6.7	6.9
2	TANK	5.3	5.9	5.5	6.0	5.5	5.2
	INNER SAP	5.0	5.0	5.0	4.8	4.7	4.4
	OUTER SAP	5.7	5.4	5.4	5.1	5.2	5.1
3	TANK	7.1	6.9	6.4	5.7	5.7	5.7
	INNER SAP	5.0	5.2	5.2	5.1	4.9	4.7
	OUTER SAP	5.7	6.6	6.5	6.3	6.3	6.2
4	TANK	7.5	7.9	7.6	7.8	8.0	7.6
	INNER SAP	5.0	5.2	5.3	5.2	4.6	4.6
	OUTER SAP	5.7	5.9	6.0	5.8	5.7	6.0

APPENDIX R 13 Preservative treatment data for non-kerfed, sprinkled Douglas fir.

Part A

IDENTITY	SPRINKLING TREATMENT AND DURATION	VOLUME (m3X10-3)	DENSITY (kg/m3)	WEIGHT BEFORE TREATMENT (g)	WEIGHT AFTER TREATMENT (g)	PRESERVATIVE UPTAKE (kg/m3)	INITIAL M.C. (%)	FINAL M.C. (%)	MAXIMUM POSSIBLE M.C.(%)	UPTAKE EFFICIENCY (%)	N.D.S.R. (kg/m3)	
4940 1	T1 2 WEEK	2.33	450	1340.5	1943.1	258	12.7	63.83	155	36	5.9	
4940 2	T1 2 WEEK	2.56	450	1428.5	2305.0	322	12.7	73.04	155	42	7.4	
4941 1	T2 2 WEEK	2.74	450	1622.9	2252.1	229	12.7	56.84	155	31	5.2	
4941 2	T2 2 WEEK	2.83	450	1686.3	2637.3	336	12.7	70.72	155	41	7.7	
4943 1	T3 2 WEEK	3.00	450	1672.1	2630.3	319	12.7	75.19	155	44	7.3	
4943 2	T3 2 WEEK	2.63	450	1393.3	2243.1	323	12.7	76.65	155	45	7.4	
4942 1	T4 2 WEEK	1.90	450	1065.0	1701.0	334	12.7	76.65	155	45	7.6	
4942 2	T4 2 WEEK	2.93	450	2035.0	3041.7	343	12.7	62.65	155	35	7.8	
4945 1	T1 3 WEEK	3.96	390	1933.9	3212.4	322	12.7	80.08	189	38	7.4	
4945 2	T1 3 WEEK	2.62	390	1285.6	2287.8	382	12.7	88.30	189	43	8.7	
4944 1	T2 3 WEEK	3.46	390	2051.9	3182.0	326	12.7	74.67	189	35	7.4	
4944 2	T2 3 WEEK	2.52	390	1212.5	1878.1	264	12.7	64.65	189	29	6.0	
4946 1	T3 3 WEEK	2.46	390	1100.4	2120.3	414	12.7	104.33	189	52	9.4	
4946 2	T3 3 WEEK	2.23	390	1233.7	2581.8	484	12.7	123.68	189	63	13.8	ESF
4947 1	T4 3 WEEK	2.93	390	1081.2	2083.0	341	12.7	113.65	189	57	11.8	
4947 2	T4 3 WEEK	3.10	390	1723.9	3353.9	525	12.7	184.79	189	52	12.0	ESF
4932 1	T1 4 WEEK	2.92	430	1705.5	2764.8	362	12.7	79.57	166	38	8.3	
4932 2	T1 4 WEEK	3.01	430	1592.1	2671.6	369	12.7	78.12	166	37	8.5	
4933 1	T2 4 WEEK	2.65	430	1520.4	2499.1	369	12.7	78.55	166	37	8.5	
4933 2	T2 4 WEEK	2.45	430	1422.5	2404.0	400	12.7	95.44	166	47	9.2	
4934 1	T3 4 WEEK	2.59	430	1359.7	2304.2	364	12.7	81.14	166	39	8.3	
4934 2	T3 4 WEEK	2.62	430	1428.2	2461.3	394	12.7	85.93	166	41	9.0	
4935 1	T4 4 WEEK	2.79	430	1540.0	2443.9	323	12.7	74.01	166	35	7.4	
4935 2	T4 4 WEEK	2.56	430	1801.9	2756.8	373	12.7	81.99	166	39	8.5	

ABBREVIATIONS N.D.S.R NET DRY SALT RETENTION USING 2.1% TANALITH NCA
M.C. MOISTURE CONTENT
ESF END-SEAL FAILURE

IDENTITY	SPRINKLING TREATMENT AND DURATION	VOLUME (m3X10-3)	DENSITY (kg/m3)	WEIGHT BEFORE TREATMENT (g)	WEIGHT AFTER TREATMENT (g)	PRESERVATIVE UPTAKE (kg/m3)	INITIAL M.C. (%)	FINAL M.C. (%)	MAXIMUM POSSIBLE M.C.(%)	UPTAKE EFFICIENCY (%)	N.D.S.R. (kg/m3)	
4928 1	6 WEEK T1	2.22	400	1191.1	2232.4	469	12.7	105.10	183	54	10.7	
4928 2	6 WEEK T1	2.41	400	1238.1	2147.0	377	12.7	82.11	183	41	8.6	
4931 1	6 WEEK T2	2.54	400	1181.7	2285.8	434	12.7	108.11	183	56	9.9	
4931 2	6 WEEK T2	2.13	400	1371.3	2646.2	598	12.7	118.77	183	62	13.7	
4930 1	6 WEEK T3	1.76	400	930.8	1867.2	532	12.7	123.22	183	65	12.2	
4930 2	6 WEEK T3	2.12	400	1041.4	2112.7	505	12.7	131.43	183	69	11.5	
4929 1	6 WEEK T4	2.01	400	1048.3	1560.3	254	12.7	72.37	183	35	5.8	
4929 2	6 WEEK T4	2.05	400	1183.6	2136.2	464	12.7	93.25	183	47	10.6	ESF
4924 1	8 WEEK T1	2.43	420	1132.5	2064.4	383	12.7	102.27	171	56	8.8	
4924 2	8 WEEK T1	2.42	420	1226.4	2227.5	413	12.7	103.46	171	57	9.5	
4927 1	8 WEEK T2	2.06	420	1311.5	2482.1	568	12.7	93.54	171	51	13.0	
4927 2	8 WEEK T2	1.98	420	1087.2	2021.9	512	12.7	123.05	171	69	11.7	
4926 1	8 WEEK T3	2.23	420	1253.6	2593.6	600	12.7	122.91	171	69	13.7	
4926 2	8 WEEK T3	2.08	420	1201.6	2432.6	592	12.7	115.79	171	65	13.5	
4925 1	8 WEEK T4	2.69	420	1595.0	3043.2	538	12.7	95.86	171	52	12.3	
4925 2	8 WEEK T4	1.79	420	1015.7	2052.4	579	12.7	119.50	171	67	13.2	
4948	CONTROL	3.77	410	1791.5	2186.5	104	12.7	28.94	177	10	2.4	
4949	CONTROL	3.39	410	1525.6	1761.1	69	12.7	38.71	177	16	1.6	
4950	CONTROL	4.27	410	2097.0	2373.1	64	12.7	26.29	177	8	1.5	

ABBREVIATIONS N.D.S.R NET DRY SALT RETENTION USING 2.1% TANALITH NCA
M.C. MOISTURE CONTENT
ESF END-SEAL FAILURE

APPENDIX R14 RADIAL PENETRATION OF CCA PRESERVATIVE INTO NON-KERFED, SPRINKLED DOUGLAS FIR

SPRINKLING TIME (WEEKS)	TREATMENT	MEAN MINIMUM RADIAL PENETRATION (mm)	MEAN MAXIMUM RADIAL PENETRATION (mm)
0	CONTROL	3	3
2	1	9	15
	2	8	26
	3	8	39
	4	9	36
3	1	13	29
	2	13	23
	3	30	38
	4	29	41
4	1	14	36
	2	17	32
	3	15	27
	4	14	15
6	1	22	34
	2	17	36
	3	33	36
	4	16	24
8	1	16	29
	2	34	36
	3	35	35
	4	35	35

APPENDIX R15 RAW DATA USED TO CALCULATE KERF WIDTH/KERF DEPTH RATIOS IN SPRINKLED AND NON-SPRINKLED DOUGLAS FIR AT EQUILIBRIUM MOISTURE CONTENT.

SPRINKLING TIME AND TREATMENT	KERF WIDTH (mm)	KERF DEPTH (mm)
NON-SPRINKLED	13.0	61.3
	10.5	59.8
	11.5	56.3
	13.0	56.3
	9.6	53.8
	11.5	54.8
	12.6	69.3
	13.0	69.3

SPRINKLING TIME AND TREATMENT		KERF WIDTH (mm)	KERF DEPTH (mm)
WEEK 2	T1	14.3	68.0
	T1	13.3	67.3
	T2	14.0	77.0
	T2	14.2	76.3
	T3	16.5	77.8
	T3	15.5	77.5
	T4	15.0	73.8
	T4	15.7	76.7
WEEK 3	T1	12.8	72.0
	T1	13.0	71.5
	T2	11.5	66.0
	T2	12.0	63.3
	T3	12.5	64.5
	T3	12.5	64.0
	T4	13.5	76.7
	T4	13.3	76.0

SPRINKLING TIME AND TREATMENT		KERF WIDTH (mm)	KERF DEPTH (mm)
WEEK 4	T1	11.3	58.5
	T1	11.3	56.5
	T2	9.3	52.3
	T2	10.0	50.0
	T3	11.7	62.0
	T3	11.0	62.0
	T4	10.5	61.5
	T4	11.8	68.7
WEEK 5	T1	14.0	78.3
	T1	13.0	78.8
	T2	13.5	78.3
	T2	13.0	76.5
	T3	13.3	70.0
	T3	13.3	68.0
	T4	13.5	63.0
	T4	13.5	62.7
WEEK 7	T1	11.0	58.6
	T1	10.5	57.8
	T2	12.5	63.4
	T2	12.5	65.0
	T3	10.8	55.0
	T3	11.0	54.4
	T4	11.3	59.0
	T4	13.3	64.4

APPENDIX R16 RAW SHRINKAGE DATA FOR DOUGLAS FIR TIMBER AFTER FIVE WEEKS SPRINKLING

TREATMENT	CUBE (REFER FIG 5-1 p.182)	E.M.C.	SHRINKAGE AT E.M.C. (%) RADIAL	TANGENTIAL	SHRINKAGE AT OVEN DRY RADIAL	TANGENTIAL
CONTROL NON-SPRINKLED REPLICATE ONE	1	14.47	2.3	4.2	4.8	8.4
	3	14.59	2.5	3.9	5.2	7.9
	4	14.23	2.2	4.8	4.5	8.3
	6	14.66	2.5	4.6	4.8	8.3
CONTROL NON-SPRINKLED REPLICATE TWO	1	14.52	2.5	3.9	4.8	8.1
	3	14.55	2.5	4.4	4.8	9.0
	4	14.58	1.6	4.6	3.8	8.3
	6	14.69	2.1	5.1	4.6	8.1
	MEAN	14.53	2.3	4.4	4.6	8.3
SPRINKLED REPLICATE ONE	1	15.11	1.8	4.1	4.4	7.2
	2	15.12	1.3	2.6	4.5	6.3
	3	14.81	1.8	3.8	4.2	7.9
	4	14.79	1.8	4.2	4.1	6.0
	5	14.91	1.8	2.6	4.7	6.9
	6	15.13	1.6	2.8	3.9	6.7
	MEAN	14.97	1.7	3.0	4.3	6.8
SPRINKLED REPLICATE TWO	1	14.84	1.5	3.0	4.6	7.3
	2	15.38	1.8	3.0	4.1	7.0
	3	15.01	2.3	2.5	4.9	6.0
	4	14.98	1.3	3.0	3.3	6.0
	5	15.13	2.0	4.0	4.9	7.2
	6	15.23	2.3	2.5	3.6	7.3
	MEAN	15.09	1.8	3.0	4.2	6.8

APPENDIX R16 CONTINUED

TREATMENT	CUBE (REFER FIG 5-1 p.182)	E.M.C.	SHRINKAGE AT E.M.C. (%) RADIAL	TANGENTIAL	SHRINKAGE AT OVEN DRY RADIAL	TANGENTIAL
SPRINKLED REPLICATE THREE	1	15.01	2.1	3.4	4.4	6.0
	2	15.01	1.6	2.9	4.2	6.8
	3	14.70	2.1	3.6	4.9	7.2
	4	14.74	1.0	1.3	4.5	6.1
	5	14.76	1.3	3.1	4.0	6.7
	6	14.97	2.6	4.1	5.2	7.8
	MEAN	14.86	1.8	3.0	4.5	6.8
SPRINKLED REPLICATE FOUR	1	14.88	1.6	3.3	3.9	6.9
	2	14.68	1.3	2.9	3.9	6.5
	3	14.48	1.6	3.1	4.5	6.4
	4	14.53	2.6	3.4	4.9	7.0
	5	14.60	1.8	3.6	4.7	7.5
	6	14.64	1.8	2.8	4.4	7.5
	MEAN	14.63	1.8	3.2	4.4	7.0

APPENDIX R17 RAW SHRINKAGE DATA FOR DOUGLAS FIR TIMBER AFTER TWELVE WEEKS SPRINKLING

TREATMENT	CUBE (REFER FIG 5-1 p.182)	E.M.C.	SHRINKAGE AT E.M.C. (%) RADIAL	SHRINKAGE AT E.M.C. (%) TANGENTIAL	SHRINKAGE AT OVEN DRY RADIAL	SHRINKAGE AT OVEN DRY TANGENTIAL
CONTROL NON-SPRINKLED REPLICATE ONE	1	8.05	2.6	5.6	4.1	8.4
	3	8.01	3.8	6.8	5.2	8.4
	4	7.82	3.8	7.0	6.2	9.1
	6	8.12	3.6	6.2	4.8	8.3
CONTROL NON-SPRINKLED REPLICATE TWO	1	7.77	3.9	6.0	5.5	8.4
	3	7.85	3.6	6.6	5.0	8.8
	4	7.80	3.6	5.9	5.0	8.1
	6	8.04	4.0	6.7	6.1	8.7
	MEAN	7.93	3.6	6.3	5.2	8.5
SPRINKLED REPLICATE ONE	1	7.47	2.8	3.4	4.0	4.4
	3	7.59	3.2	4.0	4.4	5.0
	4	8.11	3.6	3.6	4.4	6.0
	6	8.20	3.4	4.4	5.2	6.8
	MEAN	7.84	3.2	3.9	4.5	5.6
SPRINKLED REPLICATE TWO	1	8.31	3.6	4.6	5.2	7.1
	3	7.98	3.2	3.9	4.9	5.8
	4	7.90	2.8	3.2	3.8	4.8
	6	7.78	3.8	4.6	5.5	6.2
	MEAN	7.99	3.3	4.1	4.8	5.9

APPENDIX R18 PART A DRYING DATA FOR NON-SPRINKLED DOUGLAS FIR CUBES

CUBE 1 AXIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999
M.C.	122.86	110.20	104.69	77.14	62.86	50.82	31.84	20.61	15.10	8.57	7.76	7.55	7.35	7.14	6.94	0.(
"E"		0.891	0.843	0.606	0.482	0.379	0.215	0.118	0.070	0.014	0.007	0.005	0.003	0.001	0.000	

CUBE 2 AXIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999.
M.C.	128.36	115.12	110.02	80.91	65.03	51.80	30.06	19.85	14.74	8.70	7.75	7.75	7.56	7.37	6.81	0.(
"E"		0.891	0.849	0.610	0.479	0.370	0.191	0.107	0.065	0.015	0.007	0.007	0.006	0.004	0.000	

CUBE 3 RADIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999.
M.C.	128.44	113.74	105.92	80.73	67.18	51.72	28.05	20.80	17.75	12.79	12.02	9.54	8.78	8.02	7.44	-0.0
"E"		0.879	0.814	0.606	0.494	0.366	0.170	0.110	0.085	0.044	0.037	0.017	0.011	0.004	0.000	

CUBE 4 RADIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999.
M.C.	128.67	112.95	106.04	80.83	66.67	51.81	31.09	22.80	18.83	13.13	10.36	9.67	8.98	8.12	7.60	0.0(
"E"		0.870	0.813	0.605	0.488	0.365	0.194	0.126	0.092	0.045	0.022	0.017	0.011	0.004	0.000	

CUBE 5 RADIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999.(
M.C.	127.38	108.74	100.97	72.62	56.12	41.17	26.21	20.19	17.28	12.43	10.10	9.51	8.74	8.16	7.57	0.0(
"E"		0.844	0.788	0.543	0.405	0.280	0.156	0.105	0.081	0.040	0.021	0.016	0.009	0.004	0.000	

CUBE 6 RADIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999.0
M.C.	127.79	110.76	104.11	79.06	63.41	49.32	31.70	24.85	20.55	13.70	10.57	9.78	9.00	8.02	7.24	0.00
"E"		0.859	0.804	0.596	0.466	0.349	0.203	0.146	0.110	0.053	0.027	0.021	0.014	0.006	0.000	

CUBE 7 RADIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999.0
M.C.	124.90	107.05	99.79	71.58	56.85	42.12	24.48	18.67	15.98	11.62	9.34	8.71	8.09	7.47	6.85	0.00
"E"		0.849	0.787	0.548	0.424	0.299	0.149	0.100	0.077	0.040	0.021	0.015	0.010	0.005	0.000	

CUBE 8 RADIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999.0
M.C.	121.88	106.07	99.63	75.00	60.85	46.14	28.31	21.14	17.83	12.87	10.11	9.56	8.64	7.90	7.17	0.00
"E"		0.862	0.806	0.591	0.468	0.340	0.184	0.122	0.092	0.049	0.025	0.020	0.012	0.006	0.000	

APPENDIX R18 PART A CONTINUED

CUBE 9 RADIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999.0
M.C.	130.18	114.00	106.51	80.47	64.50	48.13	28.99	21.10	17.55	12.23	9.47	8.88	8.09	7.50	6.90	-0.00
"E"		0.869	0.808	0.597	0.467	0.334	0.179	0.115	0.086	0.043	0.020	0.016	0.009	0.004	0.000	

CUBE 10 RADIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999.0
M.C.	125.98	110.74	104.49	82.42	68.95	55.27	35.16	25.20	20.31	13.67	10.35	9.77	8.79	7.81	7.23	0.00
"E"		0.872	0.819	0.633	0.520	0.405	0.235	0.151	0.110	0.054	0.026	0.021	0.013	0.004	0.000	

CUBE 11 TANGENTIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999.0
M.C.	125.90	110.84	104.62	81.73	68.47	55.22	36.95	29.32	25.10	17.27	12.65	11.45	9.84	8.43	7.43	-0.00
"E"		0.873	0.820	0.627	0.515	0.403	0.249	0.185	0.149	0.083	0.044	0.033	0.020	0.008	0.000	

CUBE 12 TANGENTIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999.0
M.C.	126.60	110.28	104.08	79.26	64.72	50.71	33.87	26.77	23.05	15.78	11.52	10.46	9.22	8.16	7.45	0.00
"E"		0.863	0.811	0.603	0.481	0.363	0.222	0.162	0.131	0.069	0.034	0.025	0.014	0.005	0.000	

CUBE 13 TANGENTIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999.0
M.C.	126.55	113.18	106.78	84.69	72.48	59.50	37.21	27.71	23.84	16.86	12.60	11.63	10.47	9.11	8.33	0.00
"E"		0.887	0.833	0.646	0.543	0.433	0.244	0.164	0.131	0.072	0.036	0.027	0.018	0.006	0.000	

CUBE 14 TANGENTIAL

DRYING TIME(h)	0.0	6.0	8.0	18.0	24.0	31.0	44.0	55.0	66.0	102.0	143.0	161.0	192.0	220.0	245.0	9999.0
M.C.	126.57	110.44	104.17	79.51	66.60	52.75	34.54	27.70	24.10	16.89	12.52	11.57	10.44	9.11	8.16	0.00
"E"		0.864	0.811	0.603	0.494	0.377	0.223	0.165	0.135	0.073	0.036	0.028	0.019	0.008	0.000	

APPENDIX R18 PART B DRYING DATA FOR NON-SPRINKLED DOUGLAS FIR CUBES

CUBE 1 RADIAL

DRYING TIME(h)	0.0	7.0	10.0	22.0	30.0	40.0	57.0	71.0	86.0	133.0	188.0	213.0	9999.0
M.C.	146.04	118.32	105.45	54.46	29.70	21.29	15.84	12.87	11.88	9.41	8.91	8.42	-0.00
"E"		0.799	0.705	0.335	0.155	0.093	0.054	0.032	0.025	0.007	0.003	0.000	

CUBE 2 RADIAL

DRYING TIME(h)	0.0	7.0	10.0	22.0	30.0	40.0	57.0	71.0	86.0	133.0	188.0	213.0	9999.0
M.C.	133.19	107.96	96.02	50.88	34.96	25.22	18.14	15.04	13.72	10.62	9.73	9.29	0.00
"E"		0.796	0.700	0.336	0.207	0.129	0.071	0.046	0.035	0.010	0.003	0.000	

CUBE 3 TANGENTIAL

DRYING TIME(h)	0.0	7.0	10.0	22.0	30.0	40.0	57.0	71.0	86.0	133.0	188.0	213.0	9999.0
M.C.	146.67	121.90	110.95	68.57	50.00	36.19	25.71	20.95	18.10	12.86	10.48	10.00	0.00
"E"		0.819	0.739	0.429	0.293	0.192	0.115	0.080	0.059	0.020	0.003	0.000	

CUBE 4 TANGENTIAL

DRYING TIME(h)	0.0	7.0	10.0	22.0	30.0	40.0	57.0	71.0	86.0	133.0	188.0	213.0	9999.0
M.C.	140.31	112.76	100.51	55.61	38.27	29.08	20.41	16.33	13.78	10.71	9.18	8.67	0.00
"E"		0.791	0.698	0.357	0.225	0.155	0.089	0.058	0.038	0.015	0.003	0.000	

CUBE 5 RADIAL

DRYING TIME(h)	0.0	7.0	10.0	22.0	30.0	40.0	57.0	71.0	86.0	133.0	188.0	213.0	9999.0
M.C.	153.77	127.14	114.57	68.34	43.72	26.13	18.09	14.07	12.56	10.55	9.55	9.05	0.00
"E"		0.816	0.729	0.410	0.240	0.118	0.062	0.034	0.024	0.010	0.003	0.000	

CUBE 6 RADIAL

DRYING TIME(h)	0.0	7.0	10.0	22.0	30.0	40.0	57.0	71.0	86.0	133.0	188.0	213.0	9999.0
M.C.	145.16	114.52	100.00	50.54	31.18	22.58	16.13	13.44	12.37	10.75	9.68	9.14	-0.00
"E"		0.775	0.668	0.304	0.162	0.098	0.051	0.031	0.023	0.011	0.003	0.000	

CUBE 7 TANGENTIAL

DRYING TIME(h)	0.0	7.0	10.0	22.0	30.0	40.0	57.0	71.0	86.0	133.0	188.0	213.0	9999.0
M.C.	134.05	108.11	96.22	54.59	39.46	29.73	21.62	17.84	15.14	11.35	9.73	9.19	0.00
"E"		0.792	0.697	0.364	0.242	0.165	0.099	0.069	0.047	0.017	0.004	0.000	

CUBE 8 TANGENTIAL

DRYING TIME(h)	0.0	7.0	10.0	22.0	30.0	40.0	57.0	71.0	86.0	133.0	188.0	213.0	9999.0
M.C.	154.02	124.14	111.49	64.94	44.25	32.18	22.99	17.82	14.94	11.49	9.77	9.20	-0.00
"E"		0.794	0.706	0.385	0.242	0.159	0.095	0.059	0.039	0.015	0.003	0.000	

APPENDIX R19 PART A DRYING DATA FOR DOUGLAS FIR CUBES SPRINKLED FOR EIGHT WEEKS WITH BUFFER

CUBE 1 RADIAL

DRYING TIME(h)	0.0	1.5	3.0	5.5	7.0	10.5	27.7	31.0	34.0	36.5	40.0	42.0	45.0	62.0	80.0	97.0
M.C.	152.97	146.61	142.80	133.90	129.66	118.64	73.31	60.59	52.97	47.46	40.68	36.02	32.20	17.37	12.71	10.17
"E"		0.957	0.931	0.871	0.842	0.767	0.460	0.374	0.322	0.284	0.239	0.207	0.181	0.080	0.048	0.031

101.0	112.5	129.0	135.5	161.0	178.0	194.5	9999
9.75	8.90	7.63	7.20	6.78	5.93	5.51	0.00
0.028	0.023	0.014	0.011	0.008	0.002	0.000	0.000

CUBE 2 TANGENTIAL

DRYING TIME(h)	0.0	1.5	3.0	5.5	7.0	10.5	27.7	31.0	34.0	36.5	40.0	42.0	45.0	62.0	80.0	97.0
M.C.	161.82	154.09	150.00	140.91	136.36	125.45	81.82	70.45	63.64	58.64	50.45	46.36	41.82	25.91	17.27	12.73
"E"		0.950	0.924	0.866	0.837	0.767	0.487	0.414	0.370	0.338	0.286	0.259	0.230	0.128	0.072	0.043

101.0	112.5	129.0	135.5	161.0	178.0	194.5	9999
12.27	10.45	8.64	8.18	6.82	6.36	5.91	-0.00
0.040	0.029	0.017	0.014	0.005	0.002	0.000	0.000

CUBE 3 RADIAL

DRYING TIME(h)	0.0	1.5	3.0	5.5	7.0	10.5	27.7	31.0	34.0	36.5	40.0	42.0	45.0	62.0	80.0	97.0
M.C.	142.34	136.69	133.06	124.19	119.76	108.87	66.13	56.05	48.79	43.95	36.69	31.45	27.42	16.53	12.10	9.68
"E"		0.959	0.932	0.868	0.835	0.756	0.444	0.371	0.318	0.282	0.229	0.191	0.162	0.082	0.050	0.032

101.0	112.5	129.0	135.5	161.0	178.0	194.5	9999
9.27	8.47	7.26	6.85	6.05	5.65	5.24	-0.00
0.029	0.023	0.014	0.011	0.005	0.002	0.000	0.000

CUBE 4 TANGENTIAL

DRYING TIME(h)	0.0	1.5	3.0	5.5	7.0	10.5	27.7	31.0	34.0	36.5	40.0	42.0	45.0	62.0	80.0	97.0
M.C.	148.84	140.93	136.74	127.44	123.26	112.09	70.70	61.86	55.81	52.09	46.51	43.26	40.47	27.44	19.07	13.95
"E"		0.944	0.915	0.850	0.820	0.742	0.451	0.389	0.346	0.320	0.281	0.258	0.239	0.147	0.088	0.052

101.0	112.5	129.0	135.5	161.0	178.0	194.5	9999
13.02	11.63	9.30	8.84	7.91	6.98	6.51	0.00
0.045	0.035	0.019	0.016	0.009	0.003	0.000	0.000

CUBE 5 RADIAL

DRYING TIME(h)	0.0	1.5	3.0	5.5	7.0	10.5	27.7	31.0	34.0	36.5	40.0	42.0	45.0	62.0	80.0	97.0
M.C.	154.86	147.86	143.97	135.02	130.74	119.84	72.76	59.92	51.75	45.91	38.52	35.02	30.74	17.90	13.23	10.51
"E"		0.953	0.927	0.866	0.838	0.764	0.448	0.361	0.306	0.267	0.217	0.194	0.165	0.078	0.047	0.028

101.0	112.5	129.0	135.5	161.0	178.0	194.5	9999
10.51	9.73	8.17	7.78	7.00	6.61	6.23	0.00
0.028	0.023	0.013	0.010	0.005	0.002	0.000	0.000

CUBE 6 TANGENTIAL

DRYING TIME(h)	0.0	1.5	3.0	5.5	7.0	10.5	27.7	31.0	34.0	36.5	40.0	42.0	45.0	62.0	80.0	97.0
M.C.	145.53	136.99	133.33	124.80	120.33	110.57	73.17	63.82	58.54	54.07	48.37	45.12	42.28	29.67	20.73	15.45
"E"		0.939	0.913	0.851	0.819	0.749	0.481	0.414	0.376	0.344	0.303	0.280	0.259	0.169	0.105	0.067

101.0	112.5	129.0	135.5	161.0	178.0	194.5	9999
14.63	12.60	9.76	9.35	7.72	6.50	6.10	0.00
0.061	0.046	0.026	0.023	0.011	0.002	0.000	0.000

CUBE 7 RADIAL

DRYING TIME(h)	0.0	1.5	3.0	5.5	7.0	10.5	27.7	31.0	34.0	36.5	40.0	42.0	45.0	62.0	80.0	97.0
M.C.	147.11	141.32	136.78	128.10	123.14	112.81	69.01	57.44	50.00	44.21	36.36	31.82	28.51	16.94	12.81	10.33
"E"		0.959	0.927	0.865	0.830	0.757	0.447	0.365	0.313	0.272	0.216	0.184	0.161	0.078	0.049	0.032

101.0	112.5	129.0	135.5	161.0	178.0	194.5	9999
9.92	9.09	7.85	7.44	6.61	5.79	5.79	0.00
0.029	0.023	0.014	0.011	0.005	0.000	0.000	0.000

CUBE 8 TANGENTIAL

DRYING TIME(h)	0.0	1.5	3.0	5.5	7.0	10.5	27.7	31.0	34.0	36.5	40.0	42.0	45.0	62.0	80.0	97.0
M.C.	146.18	138.55	134.54	125.70	120.88	110.84	71.49	63.05	57.43	53.41	47.79	44.18	41.37	28.51	20.08	14.46
"E"		0.946	0.917	0.854	0.819	0.748	0.467	0.407	0.367	0.338	0.298	0.272	0.252	0.160	0.100	0.060

101.0	112.5	129.0	135.5	161.0	178.0	194.5	9999
14.06	12.05	10.04	9.24	7.63	6.43	6.02	-0.00
0.057	0.043	0.028	0.022	0.011	0.002	0.000	0.000

APPENDIX R19 PART B DRYING DATA FOR DOUGLAS FIR CUBES SPRINKLED WITH NITROGEN FOR 8 WEEKS

CUBE 1 RADIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	9.8	11.2	15.4	30.9	35.1	37.9	40.7	46.3	49.1	66.0
M.C.	157.27	150.22	145.37	139.65	133.48	128.19	116.30	110.13	92.07	34.36	25.55	22.47	20.70	17.62	16.74	13.22
"E"		0.953	0.921	0.883	0.843	0.808	0.729	0.688	0.569	0.187	0.128	0.108	0.096	0.075	0.070	0.046

84.2	101.1	105.3	116.6	132.7	139.7	178.0	213.0	299.0	9999.0
10.57	9.25	8.81	7.93	7.05	7.05	6.17	6.17	6.17	0.00
0.029	0.020	0.017	0.011	0.005	0.005	0.000	0.000	0.000	0.000

CUBE 2 TANGENTIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	9.8	11.2	15.4	30.9	35.1	37.9	40.7	46.3	49.1	66.0
M.C.	155.22	146.77	141.79	135.32	129.35	123.38	111.44	104.98	89.05	44.28	37.81	35.32	32.84	28.36	26.37	18.91
"E"		0.943	0.909	0.866	0.826	0.785	0.705	0.661	0.554	0.252	0.208	0.191	0.174	0.144	0.131	0.080

84.2	101.1	105.3	116.6	132.7	139.7	178.0	213.0	299.0	9999.0
13.93	11.44	10.95	9.45	8.46	7.96	7.46	6.97	6.97	-0.00
0.047	0.038	0.026	0.016	0.010	0.006	0.003	0.000	0.000	0.000

CUBE 3 RADIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	9.8	11.2	15.4	30.9	35.1	37.9	40.7	46.3	49.1	66.0
M.C.	149.34	141.85	137.00	131.28	123.79	118.50	105.29	98.24	83.26	28.19	22.03	20.26	18.50	16.30	15.42	12.33
"E"		0.948	0.914	0.874	0.822	0.785	0.693	0.644	0.540	0.156	0.113	0.101	0.089	0.073	0.067	0.046

84.2	101.1	105.3	116.6	132.7	139.7	178.0	213.0	299.0	9999.0
10.13	8.81	8.37	7.49	7.05	6.61	6.17	6.17	5.73	0.00
0.030	0.021	0.018	0.012	0.009	0.006	0.003	0.003	0.000	0.000

CUBE 4 TANGENTIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	9.8	11.2	15.4	30.9	35.1	37.9	40.7	46.3	49.1	66.0
M.C.	162.96	153.70	149.07	143.06	137.04	131.94	120.83	114.81	100.46	54.63	44.91	39.81	36.11	30.09	28.24	20.83
"E"		0.940	0.911	0.872	0.833	0.801	0.729	0.690	0.598	0.304	0.241	0.208	0.185	0.146	0.134	0.086

84.2	101.1	105.3	116.6	132.7	139.7	178.0	213.0	299.0	9999.0
15.28	12.50	12.04	10.65	9.26	8.80	7.87	7.87	7.41	0.00
0.050	0.032	0.029	0.020	0.011	0.008	0.002	0.002	0.000	0.000

CUBE 5 RADIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	9.8	11.2	15.4	30.9	35.1	37.9	40.7	46.3	49.1	66.0
M.C.	165.45	156.36	151.82	145.45	139.09	133.18	121.36	114.55	97.27	43.18	32.73	26.36	22.73	18.64	17.27	13.64
"E"		0.943	0.915	0.875	0.835	0.798	0.724	0.681	0.573	0.234	0.168	0.128	0.105	0.079	0.071	0.048

84.2	101.1	105.3	116.6	132.7	139.7	178.0	213.0	299.0	9999.0
10.45	9.55	9.09	8.18	7.27	6.82	6.36	6.36	5.91	-0.00
0.028	0.022	0.019	0.014	0.008	0.005	0.002	0.002	0.000	0.000

CUBE 6 TANGENTIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	9.8	11.2	15.4	30.9	35.1	37.9	40.7	46.3	49.1	66.0
M.C.	138.00	129.60	125.20	120.40	114.40	110.00	99.60	94.00	80.80	40.00	32.40	29.20	26.80	22.40	20.80	15.20
"E"		0.937	0.903	0.867	0.822	0.789	0.710	0.668	0.568	0.260	0.202	0.178	0.160	0.127	0.115	0.072

84.2	101.1	105.3	116.6	132.7	139.7	178.0	213.0	299.0	9999.0
11.40	9.60	9.20	8.00	7.60	7.20	6.40	6.00	5.60	0.00
0.045	0.030	0.027	0.018	0.015	0.012	0.006	0.003	0.000	0.000

CUBE 7 RADIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	9.8	11.2	15.4	30.9	35.1	37.9	40.7	46.3	49.1	66.0
M.C.	153.27	145.79	140.65	134.11	127.10	121.50	107.94	101.40	85.98	31.78	23.36	20.56	19.16	16.36	15.42	12.15
"E"		0.949	0.915	0.870	0.823	0.785	0.693	0.649	0.544	0.177	0.120	0.101	0.091	0.072	0.066	0.044

84.2	101.1	105.3	116.6	132.7	139.7	178.0	213.0	299.0	9999.0
9.81	8.41	7.94	7.48	6.54	6.54	5.61	6.07	5.61	0.00
0.028	0.019	0.015	0.012	0.006	0.006	0.000	0.003	0.000	0.000

CUBE 8 TANGENTIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	9.8	11.2	15.4	30.9	35.1	37.9	40.7	46.3	49.1	66.0
M.C.	150.23	143.32	138.25	131.80	124.88	118.89	106.45	100.00	83.87	41.01	35.48	33.18	30.88	26.73	25.35	17.97
"E"		0.952	0.917	0.872	0.824	0.783	0.696	0.652	0.540	0.243	0.204	0.188	0.173	0.144	0.134	0.083

84.2	101.1	105.3	116.6	132.7	139.7	178.0	213.0	299.0	9999.0
13.36	10.60	10.14	8.76	7.83	7.37	6.45	6.45	5.99	0.00
0.051	0.031	0.028	0.019	0.012	0.009	0.003	0.003	0.000	0.000

APPENDIX R19 PART C DRYING DATA FOR DOUGLAS FIR CUBES SPRINKLED WITH NITROGEN AND BUFFER FOR 8 WEEKS

CUBE 1 RADIAL

DRYING TIME(h)	0.0	1.4	4.2	5.6	8.4	25.3	26.7	29.5	30.9	32.3	33.7	37.9	40.7	43.5	59.7	77.9
M.C.	133.74	125.93	116.05	109.88	98.77	51.44	46.50	39.09	34.16	30.86	26.75	21.81	20.16	18.52	13.58	11.11
"E"		0.939	0.861	0.813	0.726	0.355	0.316	0.258	0.219	0.194	0.161	0.123	0.110	0.096	0.058	0.038

95.5	101.8	110.9	128.5	134.8	160.1	191.0	224.0	9999.0
9.47	9.47	8.64	7.82	7.82	7.00	6.17	6.17	0.00
0.025	0.025	0.019	0.012	0.012	0.006	0.000	0.000	0.000

CUBE 2 TANGENTIAL

DRYING TIME(h)	0.0	1.4	4.2	5.6	8.4	25.3	26.7	29.5	30.9	32.3	33.7	37.9	40.7	43.5	59.7	77.9
M.C.	142.86	136.55	127.31	121.85	110.92	66.81	62.61	56.30	53.78	51.26	47.90	42.44	38.66	36.55	26.05	18.91
"E"		0.954	0.886	0.846	0.765	0.441	0.410	0.364	0.346	0.327	0.302	0.262	0.235	0.219	0.142	0.089

95.5	101.8	110.9	128.5	134.8	160.1	191.0	224.0	9999.0
14.71	13.87	12.18	10.08	9.66	7.98	7.14	6.72	0.00
0.058	0.052	0.040	0.024	0.021	0.009	0.003	0.000	0.000

CUBE 3 RADIAL

DRYING TIME(h)	0.0	1.4	4.2	5.6	8.4	25.3	26.7	29.5	30.9	32.3	33.7	37.9	40.7	43.5	59.7	77.9
M.C.	138.15	132.13	122.89	117.27	106.43	59.84	54.22	45.38	40.96	37.75	32.53	24.10	20.48	18.88	12.85	10.04
"E"		0.955	0.885	0.842	0.761	0.409	0.367	0.300	0.267	0.242	0.203	0.139	0.112	0.100	0.054	0.033

95.5	101.8	110.9	128.5	134.8	160.1	191.0	224.0	9999.0
8.43	8.43	7.63	6.43	6.43	5.62	5.22	5.62	0.00
0.021	0.021	0.015	0.006	0.006	0.000	-0.003	0.000	0.000

CUBE 4 TANGENTIAL

DRYING TIME(h)	0.0	1.4	4.2	5.6	8.4	25.3	26.7	29.5	30.9	32.3	33.7	37.9	40.7	43.5	59.7	77.9
M.C.	153.85	146.58	137.61	133.33	123.50	79.49	75.21	68.38	64.96	62.39	58.55	51.71	47.86	44.02	27.78	19.23
"E"		0.950	0.889	0.860	0.793	0.493	0.464	0.417	0.394	0.376	0.350	0.303	0.277	0.251	0.140	0.081

95.5	101.8	110.9	128.5	134.8	160.1	191.0	224.0	9999.0
14.53	14.18	12.39	9.83	10.26	8.55	7.26	7.26	0.00
0.049	0.046	0.035	0.017	0.020	0.008	0.000	0.000	0.000

CUBE 5 RADIAL

DRYING TIME(h)	0.0	1.4	4.2	5.6	8.4	25.3	26.7	29.5	30.9	32.3	33.7	37.9	40.7	43.5	59.7	77.9
M.C.	148.79	140.10	129.95	124.15	113.04	67.63	63.29	56.04	52.17	49.76	46.38	40.58	37.20	33.82	19.32	14.01
"E"		0.939	0.868	0.828	0.750	0.432	0.402	0.351	0.324	0.307	0.284	0.243	0.220	0.196	0.094	0.057

95.5	101.8	110.9	128.5	134.8	160.1	191.0	224.0	9999.0
11.11	11.11	9.18	8.21	8.21	6.76	6.28	5.80	0.00
0.037	0.037	0.023	0.016	0.016	0.006	0.003	0.000	0.000

CUBE 6 TANGENTIAL

DRYING TIME(h)	0.0	1.4	4.2	5.6	8.4	25.3	26.7	29.5	30.9	32.3	33.7	37.9	40.7	43.5	59.7	77.9
M.C.	142.26	135.98	126.78	121.76	111.72	71.97	67.78	62.34	59.41	57.32	53.97	48.12	44.77	42.26	30.54	22.59
"E"		0.953	0.885	0.848	0.773	0.478	0.447	0.407	0.385	0.370	0.345	0.301	0.276	0.258	0.171	0.112

95.5	101.8	110.9	128.5	134.8	160.1	191.0	224.0	9999.0
17.57	16.32	14.23	12.13	11.30	9.62	7.95	7.53	0.00
0.074	0.065	0.049	0.034	0.028	0.015	0.003	0.000	0.000

CUBE 7 RADIAL

DRYING TIME(h)	0.0	1.4	4.2	5.6	8.4	25.3	26.7	29.5	30.9	32.3	33.7	37.9	40.7	43.5	59.7	77.9
M.C.	150.23	144.29	134.25	128.77	117.35	68.04	63.01	55.25	51.60	47.95	43.38	34.70	29.68	26.03	17.35	13.70
"E"		0.959	0.889	0.850	0.771	0.427	0.392	0.338	0.312	0.287	0.255	0.194	0.159	0.134	0.073	0.047

95.5	101.8	110.9	128.5	134.8	160.1	191.0	224.0	9999.0
11.42	11.42	10.96	10.05	8.68	8.68	7.31	6.85	0.00
0.031	0.031	0.028	0.022	0.012	0.012	0.003	0.000	0.000

CUBE 8 TANGENTIAL

DRYING TIME(h)	0.0	1.4	4.2	5.6	8.4	25.3	26.7	29.5	30.9	32.3	33.7	37.9	40.7	43.5	59.7	77.9
M.C.	137.83	130.87	121.74	117.39	107.83	66.96	63.04	57.83	54.35	52.17	48.70	42.61	38.26	34.78	22.61	16.09
"E"		0.947	0.877	0.844	0.772	0.460	0.430	0.391	0.364	0.348	0.321	0.275	0.242	0.215	0.123	0.072

95.5	101.8	110.9	128.5	134.8	160.1	191.0	224.0	9999.0
12.61	11.74	10.43	9.13	9.13	7.83	6.96	6.52	0.00
0.046	0.039	0.029	0.019	0.019	0.009	0.003	0.000	0.000

APPENDIX R19 PART D DRYING DATA FOR DOUGLAS FIR CUBES SPRINKLED WITH WATER ONLY FOR 8 WEEKS

CUBE 1 RADIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	8.4	11.2	12.6	16.8	32.3	36.5	39.3	42.1	47.7	50.5
M.C.	145.00	135.00	127.73	121.82	114.55	108.18	102.27	91.36	85.45	70.45	28.18	23.18	20.91	19.09	16.82	15.45
		0.928	0.876	0.834	0.782	0.736	0.694	0.616	0.573	0.466	0.163	0.127	0.111	0.097	0.081	0.071

67.4	85.6	102.5	106.7	117.3	134.8	141.0	166.4	200.9	233.9	9999.0
11.82	9.55	8.18	8.18	7.27	6.36	6.36	5.45	5.45	5.45	0.00
0.045	0.029	0.019	0.019	0.013	0.006	0.006	0.000	0.000	0.000	0.000

CUBE 2 TANGENTIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	8.4	11.2	12.6	16.8	32.3	36.5	39.3	42.1	47.7	50.5
M.C.	140.00	130.64	123.40	118.30	112.77	108.09	104.26	95.74	91.06	81.28	49.79	44.26	41.28	38.72	34.47	32.34
"E"		0.930	0.877	0.839	0.797	0.763	0.734	0.671	0.636	0.563	0.329	0.288	0.266	0.247	0.215	0.199

67.4	85.6	102.5	106.7	117.3	134.8	141.0	166.4	200.9	233.9	9999.0
22.98	15.74	11.91	11.49	9.79	8.09	7.66	6.38	5.53	5.53	0.00
0.130	0.075	0.047	0.044	0.031	0.019	0.015	0.006	0.000	0.000	0.000

CUBE 3 RADIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	8.4	11.2	12.6	16.8	32.3	36.5	39.3	42.1	47.7	50.5
M.C.	160.19	150.00	142.72	136.41	128.64	121.84	116.50	105.34	100.00	83.50	37.86	28.16	23.79	21.36	17.96	16.50
"E"		0.934	0.887	0.846	0.796	0.752	0.718	0.646	0.611	0.505	0.210	0.147	0.119	0.103	0.081	0.072

67.4	85.6	102.5	106.7	117.3	134.8	141.0	166.4	200.9	233.9	9999.0
12.62	10.19	8.25	7.77	7.28	6.80	6.31	5.83	5.34	5.34	0.00
0.047	0.031	0.018	0.015	0.012	0.009	0.006	0.003	0.000	0.000	0.000

CUBE 4 TANGENTIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	8.4	11.2	12.6	16.8	32.3	36.5	39.3	42.1	47.7	50.5
M.C.	151.38	140.37	131.19	125.23	118.35	112.84	108.26	97.71	92.66	79.82	39.91	32.57	28.44	26.61	22.48	20.64
"E"		0.925	0.862	0.821	0.774	0.736	0.704	0.632	0.597	0.509	0.236	0.186	0.157	0.145	0.116	0.104

67.4	85.6	102.5	106.7	117.3	134.8	141.0	166.4	200.9	233.9	9999.0
14.22	10.55	9.17	8.72	7.80	6.88	6.88	5.96	5.50	5.50	0.00
0.059	0.034	0.025	0.022	0.015	0.009	0.009	0.003	0.000	0.000	0.000

CUBE 5 RADIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	8.4	11.2	12.6	16.8	32.3	36.5	39.3	42.1	47.7	50.5
M.C.	145.19	134.13	124.52	119.23	112.02	105.77	100.96	89.90	83.65	67.79	26.44	20.19	18.27	16.83	15.38	13.94
"E"		0.921	0.853	0.815	0.764	0.719	0.685	0.606	0.562	0.449	0.154	0.110	0.095	0.085	0.075	0.065

67.4	85.6	102.5	106.7	117.3	134.8	141.0	166.4	200.9	233.9	9999.0
10.58	8.65	7.21	6.73	6.25	5.77	5.29	4.33	4.33	4.81	0.00
0.041	0.027	0.017	0.013	0.010	0.006	0.003	-0.003	-0.003	0.000	0.000

CUBE 6 TANGENTIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	8.4	11.2	12.6	16.8	32.3	36.5	39.3	42.1	47.7	50.5
M.C.	164.73	154.11	145.41	139.13	132.85	126.09	121.74	110.15	104.35	90.34	44.44	36.71	33.33	30.92	26.57	24.64
"E"		0.934	0.879	0.840	0.801	0.758	0.731	0.659	0.622	0.535	0.248	0.199	0.178	0.163	0.136	0.124

67.4	85.6	102.5	106.7	117.3	134.8	141.0	166.4	200.9	233.9	9999.0
16.43	11.59	9.18	8.70	7.73	6.76	6.28	5.31	4.83	4.83	0.00
0.072	0.042	0.027	0.024	0.018	0.012	0.009	0.003	0.000	0.000	0.000

CUBE 7 RADIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	8.4	11.2	12.6	16.8	32.3	36.5	39.3	42.1	47.7	50.5
M.C.	157.08	146.70	138.21	132.08	125.00	119.34	114.15	102.83	96.78	81.60	27.36	21.23	18.87	17.45	15.09	13.68
"E"		0.932	0.876	0.836	0.789	0.752	0.718	0.644	0.604	0.505	0.149	0.108	0.092	0.083	0.068	0.058

67.4	85.6	102.5	106.7	117.3	134.8	141.0	166.4	200.9	233.9	9999.0
10.38	8.49	7.08	7.08	5.66	5.66	5.19	4.72	4.25	4.72	0.00
0.037	0.024	0.015	0.015	0.006	0.006	0.003	0.000	-0.003	0.000	0.000

CUBE 8 TANGENTIAL

DRYING TIME(h)	0.0	1.4	2.8	4.2	5.6	7.0	8.4	11.2	12.6	16.8	32.3	36.5	39.3	42.1	47.7	50.5
M.C.	171.58	161.05	152.63	144.74	135.26	127.37	121.05	107.89	101.58	84.21	48.95	43.68	41.05	38.42	33.68	31.05
"E"		0.937	0.886	0.838	0.781	0.733	0.695	0.616	0.578	0.473	0.260	0.229	0.213	0.197	0.168	0.152

67.4	85.6	102.5	106.7	117.3	134.8	141.0	166.4	200.9	233.9	9999.0
22.11	15.26	11.58	10.53	9.47	8.42	7.89	6.32	5.79	5.79	0.00
0.098	0.057	0.034	0.028	0.022	0.015	0.012	0.003	0.000	0.000	0.000

diagram to accompany Thesis by Kevin John Archer "Bacterial Modification of Douglas Fir Roundwood Pe

BACTERIAL MODIFICATION OF DOUGLAS FIR ROUNDWOOD PERMEABILITY

K. Archer, School of Forestry,
University of Canterbury.

BACTERIAL MODIFICATION

OF DOUGLAS FIR ROUNDWOOD PERMEABILITY

The purpose of this study is to attempt to improv
preservative in Douglas fir roundwood thinnings, thereby creating a market for a largely wasted resource.

Over the last 25 years water storage of different tree species has been known to improve permeability. Bacteria colonizing the wood in storage have been shown to be responsible for the phenomenon.

This study aims to optimize the conditions necessary for successful bacterial colonization and hence to optimize permeability improvement.

25 year old Douglas fir trees were felled at Ashley Forest, Canterbury and brought back to the School of Forestry. The trees were cut into 2 metre lengths, sealed in plastic bags and stored at 4^{o} C.

When required, the logs were debarked and quartered. Pieces from each log were numbered and allocated randomly to one of six groups. Four of the groups were incised to a depth of 2 cm on the tangential face only. One incised and one non-incised group were set aside as controls. The remaining 4 groups were placed into separate sprinkling tanks.

The six treatments can be summarized as follows :

1) Incised, no sprinkling;
2) Incised, cyclic sprinkling - no nutrients;
3) Incised, cyclic sprinkling - nutrients;
4) Incised, constant sprinkling - nutrients;
5) No incising, cyclic sprinkling - nutrients;
6) No incising, no sprinkling.

The nutrient solution consisted of the following :

K_2HPO_4	306g
KH_2PO_4	72g
$MgSO_4$	9g
$(NH_4)_2SO_4$	90g
NaCl	9g
$FeSO_4$	trace
$CaCl_2$	1.8g

In 18 litres of distilled water.

The sprinkling cycle involved 2 hours of sprinkling, with one hour off. All four tanks were inoculated with a mixed culture of bacteria isolated from water stored Douglas fir. The bacteria had been previously tested for their ability to improve the permeability of small cubes in shake culture.

The experiment was terminated after 8 weeks and the samples kiln dried. A mild kiln schedule was selected to minimize the possibility of checking (35° C dry bulb, 30° C wet bulb giving 70% r.h.). Before drying the pieces were endsealed with three coats of "epiglass reaction lacquer". Weight losses were recorded daily. As soon as the moisture content for each treatment group had fallen below 28%, the entire group was removed to a conditioning room and conditioned for 7 days. A standard Bethel treatment with an initial vacuum of -85kPa for 30 min. followed by a pressure cycle of 1385kPa for 2 h until refusal was used. No final vacuum was pulled. After removal from the cylinder the samples were allowed to drip dry before weighing. Per cent gain in weight was calculated for each sample in each treatment and then averaged. Samples were then cut in half and the depth of penetration ascertained with chemical developing reagents. Copper was detected with chrome azurol, arsenic with sodium molybdate/$SnCl_2$ and chromium with silver nitrate.

RESULTS

Drying

Drying curves for each treatment are presented below in figure 1. Sprinkling treatment increases the moisture content of the timber by up to 30%. All sprinkled treatments took longer to reach fibre saturation point than the controls. Treatment 2 took 2 days longer than the controls to reach f.s.p. and treatments 3, 4 and 5 took 4 days longer.

The exposed surfaces of treatments 3, 4 and 5 became covered with a white powdery deposit as they dried. The deposit was absent on the surface of treatment 2. Treatments 3, 4 and 5 showed less of a tendency to check on drying than treatments 1 and 6. Treatment 2 showed a checking tendency somewhere in between that of 3, 4 and 5 but the observation was not quantified. Some biscuits cut from the logs after sprinkling to determine moisture contents showed an orange stain in the sapwood.

Treatment

The effect of the various treatments on the uptake of preservative is compared in figure 2. The average per cent gain in weight is presented for each treatment. It is obvious from comparing the controls and sprinkled treatments that there is an improvement in the uptake of CCA in the latter. Treatment 5 absorbed 3.5 times more CCA than the non-incised control, treatment 6. The incised treatments 2, 3 and 4 did not show such a large increase, the increase being only twice that of the incised control. Treatments 3 and 4 show the different effects of cyclic and constant sprinkling. CCA uptake was higher in treatment 4 than in treatment 3. An interesting point to note is that the variability in uptake between individual bolts in treatment 3 is smaller than that in treatment 4.

The presence or absence of nutrients does not seem to affect the uptake of CCA when we compare treatment 2 and treatment 3.

The depth of penetration of CCA and its various components is shown in table 1.

The data in table 1 show that while the uptake of fluid is increased in sprinkled treatments, the relative uptake of the individual CCA components differs. Copper is heavily screened and penetrates only a short distance; arsenic travels further, while chromium achieves the greatest penetration.

DISCUSSION

The increased drying time required by treatments 3, 4 and 5 is significant. A similar phenomenon was noted by Boutelje in 1976 and more recently by Martin at the 1981 British Wood Preservers' Annual Meeting. Boutelje observed that water sprinkled Scots pine was not sufficiently dry to treat after one and a half years air drying. Neither Boutelje nor Martin adequately explained the reasons for the poor drying and the phenomenon requires further investigation.

CCA uptake has definitely been improved by sprinkling, but the results have raised more questions than they have answered. The results presented for the penetration of various CCA components are based on spot tests only and thus require more rigorous chemical analysis. The disproportionation phenomenon is a problem with Douglas fir and has already been noted in previous studies by the F.R.I. The influence of bacteria on disproportionation will be examined at a later date. It should be stressed at this point that the conditions necessary for improvement of permeability may not have been optimized. The question of optimization is central to this study and will be further investigated.

FIGURE 1. DRYING CURVES FOR THE SIX TREATMENTS

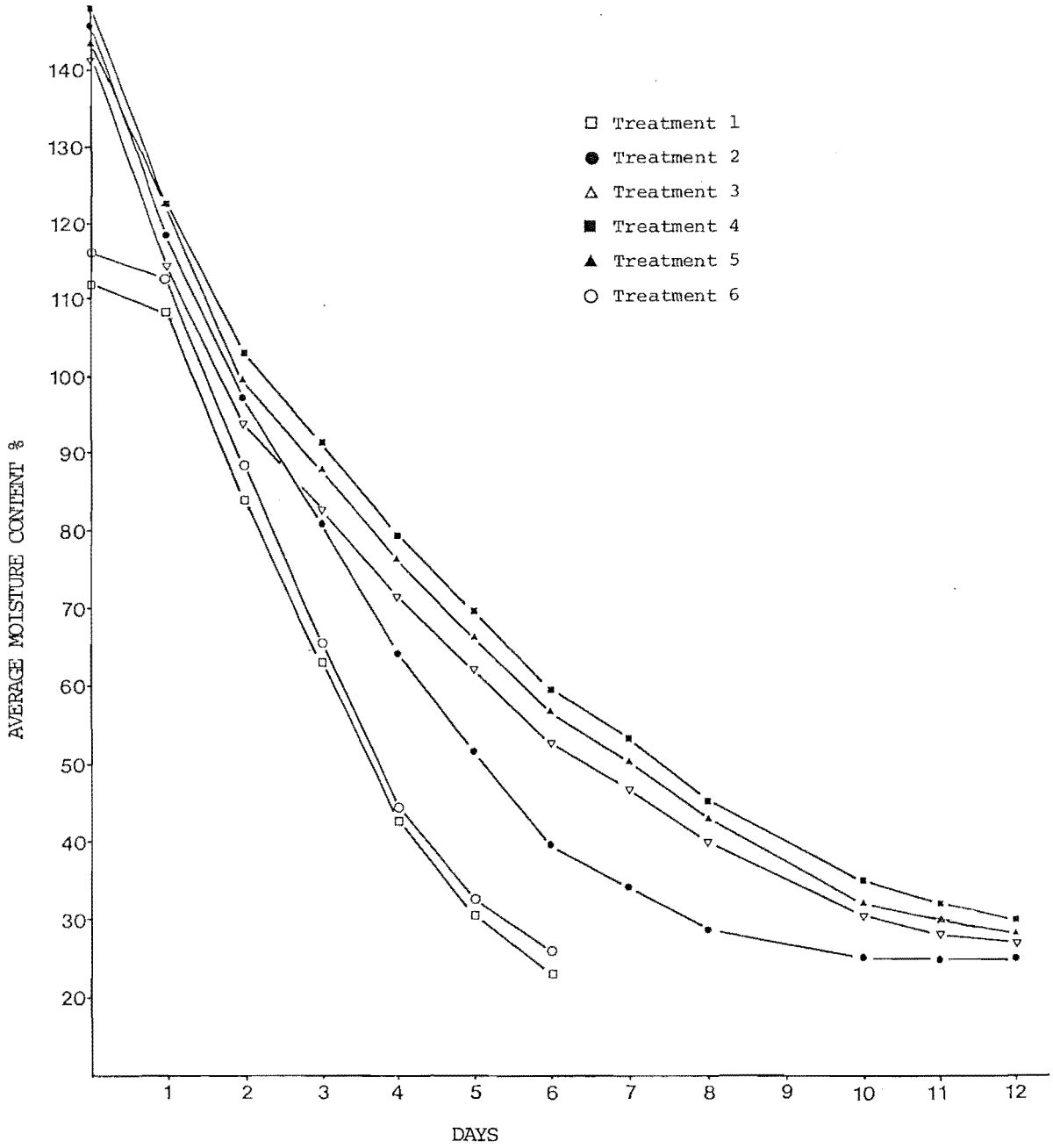


FIGURE 2. MEAN PERCENTAGE UPTAKE FOR THE SIX TREATMENTS

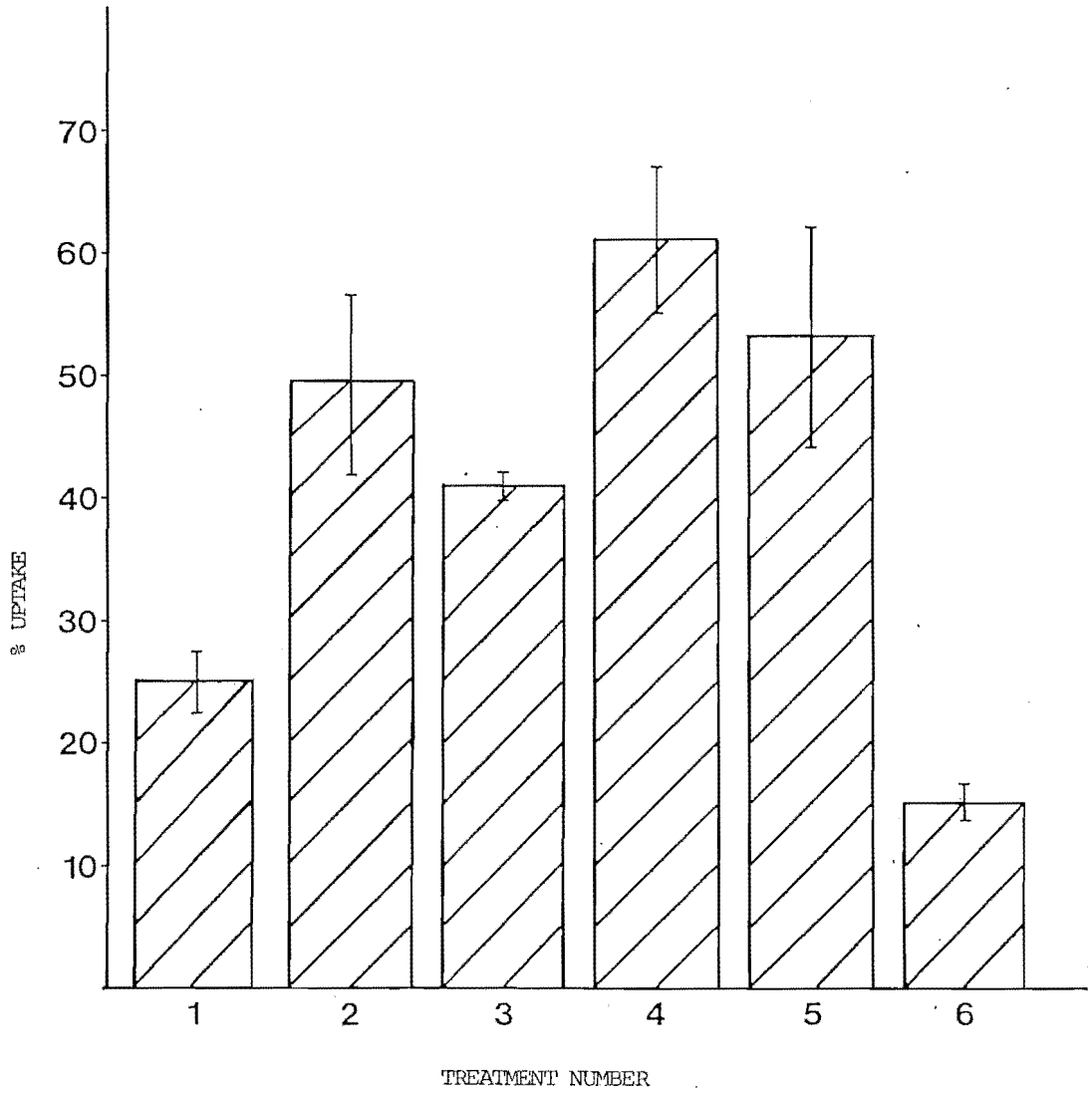
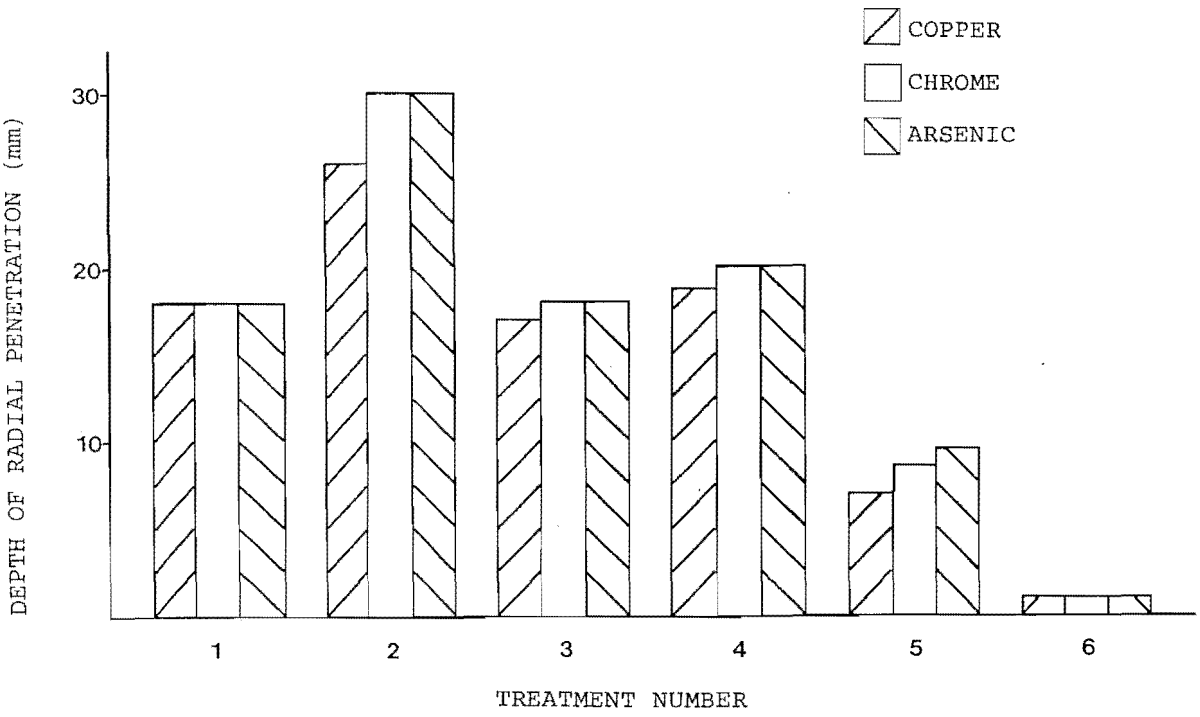
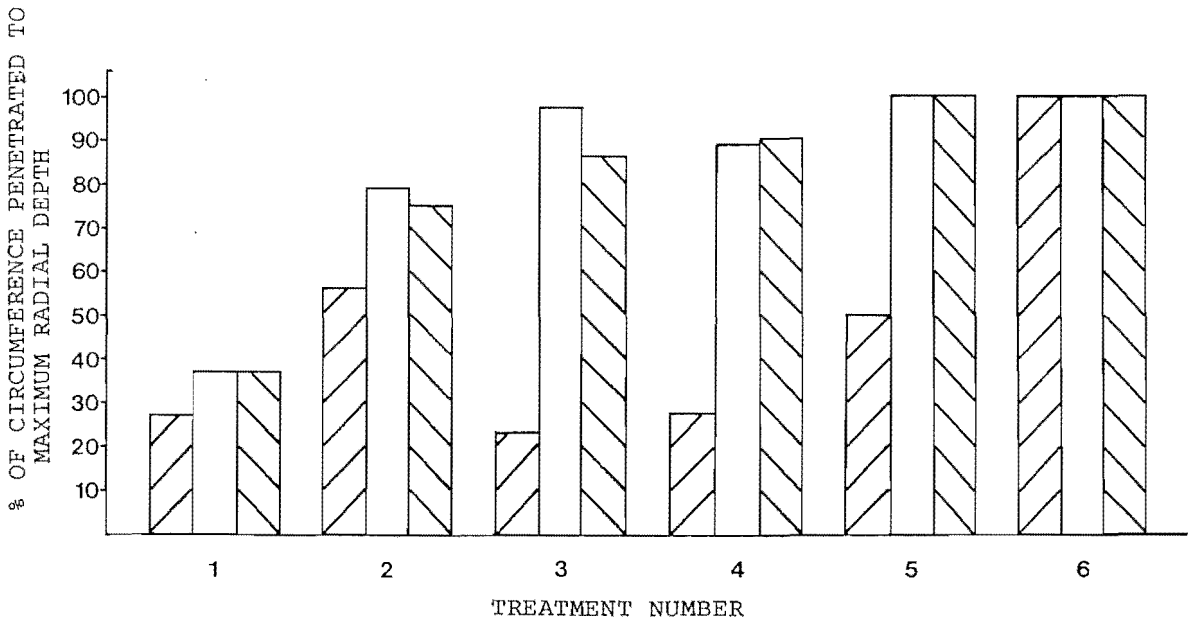


TABLE 1. PENETRATION OF CCA COMPONENTS





THE USE OF BACTERIA TO IMPROVE THE UPTAKE OF PRESERVATIVE IN DOUGLAS FIR ROUNDWOOD

K.J. Archer
School of Forestry
University of Canterbury

In N.Z. Douglas fir is the second most widely planted exotic species after radiata pine and in 1982 comprised 8 % of the total timber cut. Under present N.Z. Forest Service silvicultural practice the cost of producing radiata saw logs is partially offset by the utilisation of production thinnings for fence posts and poles. However Douglas fir thinnings are not normally harvested mainly because they do not command a ready market. One important reason for this is that Douglas fir is only moderately durable and needs preservative treatment for use in high decay hazard situations (eg. in ground contact). Douglas fir is however regarded as being impermeable, which is unfortunate because in many ways Douglas fir is better suited to pole use than pine because of its higher tensile strength and better form. In N.Z. the most commonly used preservatives are of the water based copper chrome arsenate (CCA) type. Douglas fir roundwood and sawn timber are not amenable to CCA treatment - penetration of preservative is poor and very patchy. The N.Z. Timber Preservation Authority (TPA) regulations for the treatment of round material in ground contact require that CCA penetrates to a minimum uniform depth of 10 mm or 75 % of the sapwood which-ever is the greater. This is seldom achieved with Douglas fir,so the end uses of the timber are limited.

Why is Douglas fir impermeable? An answer to this question has stimulated many years of research and debate and is still in dispute. Anatomical factors are generally held responsible. Conduction of preservative in axial and tangential directions is controlled by bordered pits. These structures function as valves in the living tree and guard against air embolisms stopping the flow of sap. In green timber the pits are normally in a central position and are open. When the timber is dried prior to preservative treatment they aspirate, effectively sealing off the tracheids and preventing the flow of fluids through wood (Bolton and Petty 1975). In practice the sealing between pit and aperture is thought to be far from perfect (Bailey 1965) and to a certain extent the pits can be displaced sufficiently to facilitate fluid flow. However the major limiting factor in the permeability of round wood material is the poor radial movement of fluids ie. from the outside in.The major radial pathways for preservative are the ray tissues. Rays are connected to the longitudinal tracheids via crossfield tracheid to ray pits. It is possible to observe the flow of dyes through minute pores in the margo of bordered pits. But there are no such pores in the tracheid to ray crossfield pits and movement of fluids is by

diffusion. In Douglas fir these pits are occluded with phenolic material and cytoplasmic debris which hinder diffusion (Liese and Bauch 1967, Bauch and Berndt 1973). These occlusions and the fact that fluids must therefore cross many cell walls contribute to the poor radial flow. This is not the case with pine and hence radial permeability in pine is far greater. Bolton and Petty (1975) were able to demonstrate that pit membranes are the main hindrance to the flow of preservatives by comparing the permeability of ponded and unponded Sitka spruce. If the pit obstructions can be removed from Douglas fir then preservative treatment is likely to be improved.

Over the years a number of methods have been tried to achieve this with only partial success. Most techniques that have been used structurally weaken the timber creating further problems for the eventual end usage. Since the late 1950's water storage of timber under sprinkling systems and in ponds has been known to improve permeability (Suolaihti and Wallen 1958, Knuth and McCoy 1962). Logs in wet storage experience complex microbial successions and interactions which are still poorly understood. Most importantly after a short period of time certain bacteria break down the intercellular pit membranes and allow the free passage of preservative solutions. It is ironic that the association of bacteria with water storage and permeability of logs was once regarded as a major problem - water stored logs were OVER porous. The selectivity of the invading micro-organisms for pits is important because it minimises strength loss - a problem with physical methods. Pit membranes contain a high proportion of pectin (Liese 1970) as well as hemicellulose and cellulose. The production of pectinases by invading bacteria with little or no cellulase activity has been shown to be the reason for selectivity in pit degradation (Fogarty and Ward 1972).

The aim of my work therefore is to examine the factors affecting the success of water storage, to attempt to control them and in so doing provide uniform improved permeability of Douglas fir to CCA.

Bacterial cultures were isolated from a Douglas fir log pile which had been kept wet under a water sprinkler for several months. Wood samples from the log pile had shown improved permeability over control samples and there was microscopic evidence of pit degradation suggesting that any bacteria isolated might be capable of improving permeability. Crude bacterial cultures were screened for pectinolytic activity using pectin agar plates. Promising cultures were set aside for further analysis. One particular culture which later proved to be a mixture of two Bacillus species, a coryneform sp. and an Enterobacter appeared particularly promising. This mixture when cultured with microtome sections of Douglas fir sapwood was able to degrade pit membranes after a few days. Tests with pectin as a sole carbon source showed that individually the bacteria were not so efficient at degrading the substrate as they were when

mixed together suggesting the possibility of synergistic growth. This observation was based on accumulation of pectin breakdown products , accumulation of reducing sugars and culture turbidity in pure and mixed cultures. All cultures were motile in 18 h glucose broth preparations. It was decided without any further analysis to use this mixed culture in experiments to improve permeability. Numerous other studies have implicated all three genera of bacteria contained in the mixture as causing permeability improvements (Knuth and McCoy 1962,Ward and Fogarty 1973).

There have been several attempts around the world to inoculate logs with bacteria known to improve permeability. For a variety of reasons these attempts have been only partially successful (Fowlie 1981). To optimise water storage conditions for permeability improvement the important environmental parameters must be established. From a general knowledge of microbial growth requirements it seems likely that the degree of aeration, temperature, nutrient supply and pH will be relevant. Other studies have shown that the production of polygalacturonate lyases (enzymes known to be involved in pit breakdown) is enhanced under conditions of high phosphate concentration ,in the presence of calcium ions and at a pH above 7 (Macken and Pickaver 1979,Kurowski and Dunleavy 1976, Fogarty and Ward 1972). It was decided to incorporate these observations into the current study and to culture Douglas fir logs with the mixed population of bacteria. in a buffered system using phosphate both as a growth factor and as the pH buffer.

To make a water storage system economically viable it is essential to effect permeability improvement as fast as possible. A primary requirement is that bacteria must first colonise the wood in sufficiently large numbers. It has been calculated that at least 1 million cells per gram of wood are needed to effect any significant change (Line 1983 pers comm.). This means that bacteria will have to be introduced into the wood as quickly as possible and in large numbers. Normal entry of bacteria in wood is passive and depends to a large extent on diffusion. The organisms must literally eat their way in - a slow process. In a natural situation this entry is typically patchy giving rise to localised areas of undegraded pits. This results in areas deficient in preservative after pressure treatment - a common complaint with water stored wood. To get around this problem and and to allow rapid bacterial entry it was decided to incise the timber opening up the wood structure to a uniform depth.

25 year old Douglas fir trees were felled in Ashley Forest, Canterbury,in late spring 1983. They were cut into 1.8 metre logs, brought back to the School of Forestry, sealed in plastic sleeving and stored in a cool store at 4 C until needed. When required the logs were debarked and quartered. Pieces from each log were numbered and randomly allocated to one of five groups. Three groups were incised. Four rows of incisions at 90 degrees to each other were made to a depth of 2.5 cm by passing the logs

FIGURE 1

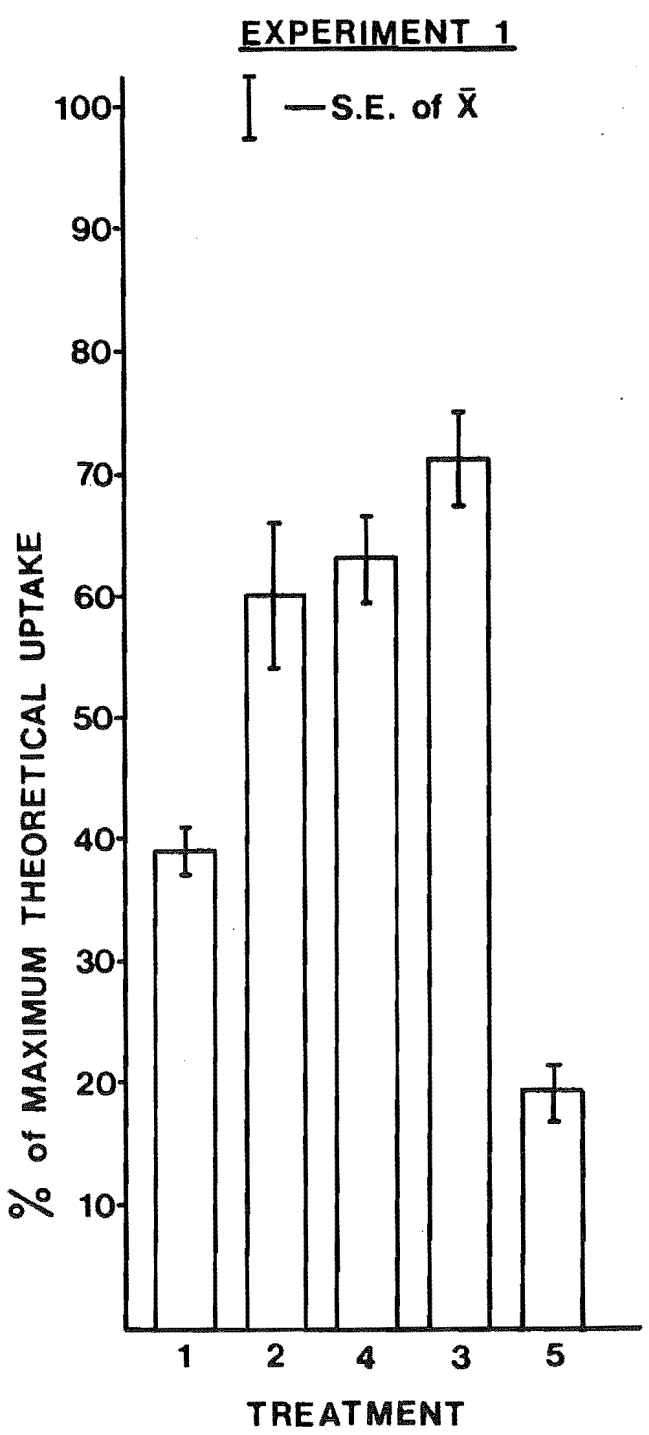
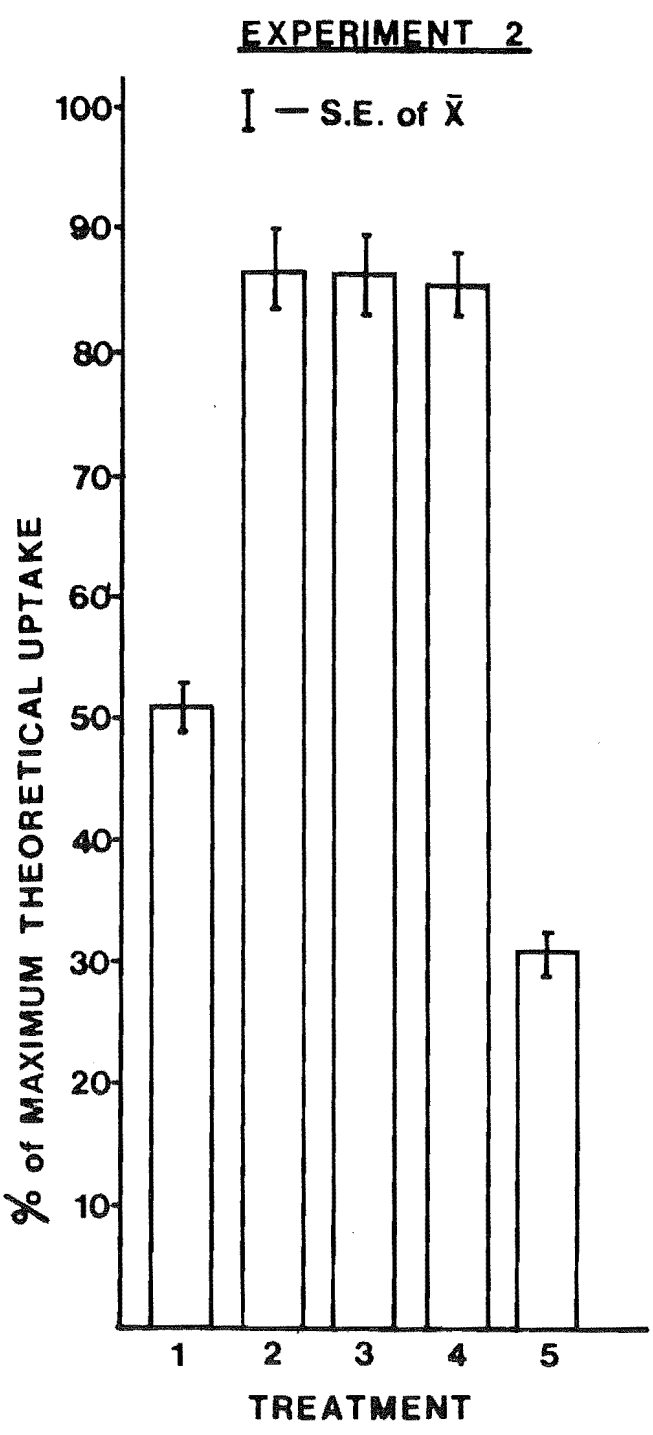


FIGURE 2



The treatments are as follows:-

1. Incised, no sprinkling, no bacteria
2. Incised, sprinkled, no nutrients + bacteria
3. Incised, sprinkled, nutrients + bacteria
4. Non incised, sprinkled, nutrients + bacteria
5. Non incised, no sprinkling, no bacteria

under a toothed roller. One incised and one non incised group were set aside as controls and the 3 remaining groups were placed in separate sprinkling tanks. The tanks were inoculated with 3 day old trypticase soy broth cultures. The five treatments can be summarised as follows:-

1). Incised,no sprinkling,no bacteria
2). Incised,sprinkled ,no nutrients + bacteria
3). Incised,sprinkled , nutrients + bacteria
4). Non incised, sprinkled, nutrients + bacteria
5). Non incised,no sprinkling,no bacteria

The nutrient solution consisted of the following:

K_2HPO_4	17g
KH_2PO_4	4g
$MgSO_4$	0.5g
$(NH_4)_2SO_4$	5g
NaCl	0.5g
$FeSO_4$	0.1g
$CaCl_2$	0.1g

per liter of distilled water, final pH 7.2.

Prevailing temperatures for the duration of the experiment ranged from 13-15 C. The experiment was terminated after eight weeks and the samples kiln dried prior to preservative treatment. When the average moisture content of the pieces had fallen below fibre saturation point they were subjected to a standard Bethell preservative treatment with an initial vacuum of -85KPa held for 30 min followed by a pressure cycle of 1385 KPa for 2 h until refusal was reached. After removal from the pressure cylinder the samples were allowed to drip dry before weighing. Percent gain in weight was calculated for each sample and then averaged for each treatment and compared. Samples were then cut in half and the average depth of penetration was determined. The effect of the various treatments on uptake of preservative is presented in figure 1. Depth of penetration and the percentage of maximum theoretical uptake possible are important parameters. It can be seen that although uptake is improved as a result of bacterial sprinkling, the improvement is far from optimal. Further-more the depth of penetration was insufficient to satisfy the TPA regulations. Incising doubled the uptake in the controls but no such difference is evident in the bacterial treatments. Since the aim of this study is to optimise the sprinkling process it was decided to examine the environmental parameters used in this experiment and check for sub-optimal levels. The most obvious fault in the system was the temperature. Banks (1970) showed that permeability of water stored Scots pine was markedly improved by increasing the temperature from 10 C to 30 C. Temperature of the sprinkling tanks in the first experiment was entirely dependent on the ambient room temperature which had a wide diurnal fluctuation but was below 15 C most of the time. Bacterial growth at this temperature was far from optimal in incubated cultures and it was therefore decided to repeat the experiment at a higher temperature. The sprinkling tanks were placed in a humidity and

temperature controlled room at 55-60 % humidity and 21 C. The results after 8 weeks sprinkling are presented in figure 2. For all practical purposes each bacterial treatment has achieved the maximum theoretical uptake possible. This is further evident when the depth of penetration is measured - 100 % sapwood penetration is obtained. This is an excellent result from the point of view of preservative treatment but in terms of shedding light on the optimal sprinkling requirements it is disappointing. It appeared that the addition of nutrients to stimulate bacterial growth or incising to aid dissemination had no effect. This was not a realistic observation and it seemed more plausible that differences between the treatments would be reflected in the amount of time it took for each treatment to achieve maximum uptake.

To investigate this possibility a further experiment was set up. Improvements in permeability between nutrient sprinkled logs and non nutrient sprinkled logs were to be examined over a period of 8 weeks. Logs were removed from each treatment and sampled for numbers of organisms per gram of wood tissue, for enzyme activities,to determine the extent of pit degradation microscopically and also to observe any differences in uptake. Changes in pH of the sprinkling solution and within the wood were also monitored. Previous studies have shown that the pH of sprinkled logs falls to around pH 5 quite rapidly (Ward and Fogarty 1973, Macken and Pickaver 1979). At the time of writing collection of data for this experiment is still in progress but some preliminary results can be mentioned. As expected the weak buffering capacity of treatment 1 held the pH of the sprinkling solution above 7 for 5 weeks. The pH of the unbuffered solution fell quite rapidly from 6.85 to 6.2 in the same period. No bacterial counts were made for the first 3 weeks but at 4 weeks the numbers in nutrient sprinkled logs were 3.5 X those of the non nutrient system. Although the data is incomplete it appears that peak numbers of bacteria occur at least 2 weeks earlier in the presence of nutrients than without them. Microscopic examination of the pit membranes in the nutrient treatment showed that after 5 weeks most of the bordered pits had been degraded to the extent that they were missing completely and there was extensive attack on the tracheid to ray cross field pits. In comparison,some bordered pits in the non-nutrient system showed signs of degradation but a large number were still intact. There was some evidence of tracheid to ray pit damage but complete solublisation of the pit membrane is not necessary for an increase in permeability to occur.

There was no measureable pectinase activity in the sprinkling liquid in either treatment but activity was found in the expressed sap from sprinkled logs. The accumulated data still require analysis hence the results are not presented here. However peak activity coincides with peak numbers as might be expected.

The differences in numbers of bacteria and in the degree of pit

degradation between nutrient and non-nutrient systems suggests that promising preservative treatment differences will be found. At this stage however uptake data are unavailable. The presence of nutrients in the sprinkling solution favours rapid bacterial growth and at the same time supresses fungal growth. Evidence for this comes from the fact that fungal colonies appeared in the dilution plates from non-nutrient sprinkled logs. This is interesting because the medium used for the dilution plates was not selective for fungi. Fungal decay in the outer layers of the timber was also evident in the non-nutrient sprinkled logs.

In conclusion this study has shown that :-

(1) the sprinkling of Douglas fir poles with a mixture of bacteria and nutrients improves the uptake of CCA preservative to the extent of 100%
(2) sprinkling of Douglas fir poles with the same bacterial mixture but without nutrients also improves CCA uptake but the process appears slower
(3) temperature is an important variable with respect to speeding up permeability improvement
(4) the addition of nutrients to the sprinkling solution stimulates bacterial growth which in turn appears to suppress fungal colonisation of the poles

It is therefore possible that biotechnology has a part to play in the more efficient use of our timber resources.